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REVIEW ARTICLE

BILE SALTS—THEIR PHYSIOLOGICAL FUNCTIONS
IN THE GASTROINTESTINAL TRACT

Bile salts and related compounds (bile alcohols) are present in the digestive tract of all vertebrates. The cooperative chemistry of these compounds has been elucidated by Haaslewood, the C_{24} acids being the latest step in the evolution (25). The bile salts belong to the class of detergents, that is to say their molecules possess both fat and water-attracting regions, which give them special properties in water solution. Other types of detergent have been found in the gastric juice of some crustaceans (crab lobster crayfish); these are composed of sordium chain fatty acids acylated to one or two amino acids, one example being the acyl-sarcosyl-taurine of the crab (37,54).

The universal occurrence of detergents in the gastrointestinal tract points to their great physiological importance in spite of which, up to the last decade, the function of the bile salts in the intestinal tract has been rather vaguely formulated as an aid in the emulsification of the dietary fat and as activators for lipase. It seems now clear however that, besides having general detergent properties, the bile salts also have certain rather specific functions in the intestinal tract which will be discussed here.

CHEMISTRY—PHYSICAL CHEMISTRY—
METABOLISM

Chemistry The bile salts are formed in the liver from cholesterol and contain the well recognized steroid skeleton (3, 5). The conversion of cholesterol to bile salts involves a series of chemical transformations the mechanism of which has been elucidated in great detail (4). The two bile acids primarily formed in man are the 3,7,12-trihydroxycholecanoic acid called cholic acid and the 3,7-dihydroxycholecanoic acid called chenodeoxycholic acid. Two secondary bile acids, 3,12-dihydroxy- and 3-monohydroxycholecanoic acid with the trivial names deoxycholic and lithocholic acid, are formed from the primary bile acids in the intestinal tract by the action of bacteria which remove the OH group in the 7 α -position, of these only deoxycholic acid is reabsorbed and excreted to the bile in measurable quantities. In the bile the bile acids are present in conjugated form

with glycine or taurine to form choyl taurine, deoxycholyglycine etc.

Physical chemistry In water solution bile salts (in the following bile salts refer to salts of the conjugated bile acids) have detergent properties (30, 36). At low concentrations they form molecular dispersed solutions. At a certain concentration that is characteristic for each bile salt the bile salt molecules, due to their content of hydrophilic and hydrophobic centers, start to form aggregates called micelles. The concentration is usually referred to as the critical micellar concentration (CMC) but in general is more of critical micellar range. Detergents added to the solution in excess of this range all form aggregates and the molecular dispersed phase which therefore has constant concentration, enters into rapid equilibrium with the micelles which form the micellar phase (Fig. 1). The size and shape of the micelles vary with the type of bile salt, the trihydroxy conjugates under physiological conditions forming aggregates with 4-8 molecules, the dihydroxy bile salts having aggregation numbers of the order of 16-25. From a physico-chemical point of view the bile salts behave in principle similarly to other anionic detergents. Bile salt solution has a much lower solubilizing capacity for non-polar lipids than ordinary paraffin chain detergents the capacity of bile salt solutions to solubilize insoluble amphiphilic lipids such as monoglycerides into mixed micelles is, on the other hand, much greater. The ability of these mixed bile salt micelles to solubilize non-polar lipids is comparable to that of the ordinary lipophilic class detergents.

Metabolism Bile acids are synthesized in the liver from cholesterol, conjugated with taurine or glycine and excreted in the bile (?). A most important feature of bile salts is that they undergo an enterohepatic circulation with rather efficient reabsorption normally better than 98% (68). The total pool of bile salt in man is of the order of 2.5-4.5 g (42, 66). When the gastrointestinal tract is at rest, this pool is almost entirely concentrated in the gallbladder and the bile secreted from the liver is low in bile salt. After food intake the gallbladder contraction forces the bile salt pool into circulation and the bile salts are reabsorbed and re-excreted in the liver bile as long as digestion takes

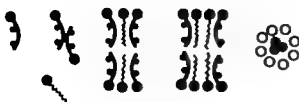


Fig 1 Structures present in a solution of bile salts above the critical micellar concentration in the presence of another amphiphilic molecule. The molecular dispersed phases are considered to be in equilibrium with the micellar phase.

place. The extent of recirculation of the bile salt pool depends on the number of meals and their size and composition and it is considered that the pool circulates 6–10 times a day (8). The absorption of bile salts is passive in the jejunum and upper ileum (mostly glycine conjugates) and active in the lower ileum (glycine and taurine conjugates). For every circulation some bile salt is normally lost to the colon. Absorption here is slight and bacterial degradation of the bile salt molecule takes place resulting in large number of different metabolites, most of which are excreted by the feces. Deconjugation and 7 α -dehydroxylation are the most important processes resulting in the formation of free lithocholic acid (3-OH cholic acid) from chenodeoxycholic acid and free deoxycholic acid (3,12-OH-cholic acid) from cholic acid.

The excretion of bile salts via the feces is around 800 mg/day giving a half life of the bile salts in the order of 3 days. Each individual bile salt molecule synthesized in the liver thus undergoes 15–20 enterohepatic circulations before it is lost via the feces (66), extent to which the bile salts are deconjugated. Normal enterohepatic circulation has been estimated to be 10%/day for the taurines (28, 63) and appreciably more for the glycine conjugates (27, 53).

The different kinetic parameters of bile salt metabolism—pool size, rate of hepatic synthesis and the enterohepatic cycling—can be measured by chemical methods and by the use of labeled bile acids. Variations in these parameters during different diseased states are of great diagnostic importance (34). The chief parameter of interest for digestion and absorption is the actual bile salt concentration in the contents of the upper small intestine during digestion which can be sampled and determined. After a peak value during the first 20–30 min after food intake due to the emptying of the gall-bladder, the recirculated liver bile gives a concentration of bile salts in the intestinal content of 4–16 mM (8, 61). This concentration is above the CMC of the mixed bile salt solution and a micellar phase is present in the intestinal content under normal conditions. The micellar phase of intestinal content can be obtained through ultracentrifugation or filtration through membrane filters (33, 54). When the bile salt concentration is decreased below this level a micellar phase defect occurs which in general results in malabsorption (57).

The synthesis of bile salts by the liver is regulated by the amounts of bile salt returning to the liver. The

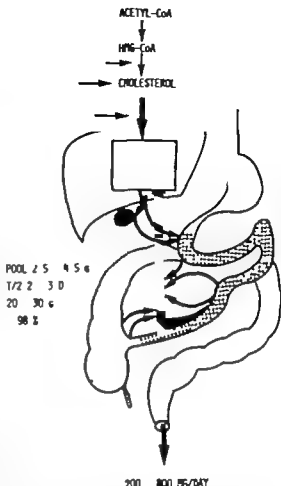


Fig 2 The metabolism of bile salts in essence. After being formed from cholesterol in the liver or reabsorbed from the blood by the liver the bile salt molecules are secreted to the bile discharged to the small intestine from which after having fulfilled their physiological function they are absorbed actively by the distal ileum to the portal circulation, reexcreted by the liver etc. The amounts of bile salts excreted by the feces, which normally constitute a minor fraction of the totally circulated bile salts are compensated for by *de novo* synthesis in the liver from cholesterol. The *de novo* synthesis of bile salts in the liver is under negative feedback control by returning bile salt.

maximum capacity of the human liver to synthesize bile salts is considered to be 4–6 times that of the normal level (21).

ROLE OF BILE SALTS FOR DIGESTION AND ABSORPTION OF FATS

The digestive enzyme

Pancreatic lipase. It has long been considered that bile salts are activators for pancreatic lipase. Recent investigations, however, have shown that bile salts rather are inhibitors of this enzyme when it is present

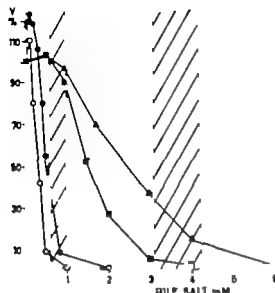


Fig. 3 Effect of bile salts on the lipase-catalyzed hydrolysis of tributyrine (V = rate of hydrolysis). Effect of two dihydroxyconjugates: chenodeoxycholytaurine (O), deoxycholytaurine (●), and two trihydroxyconjugates: cholestyramine (■) cholestyramine (A), B = the critical micellar range of the di- and tri-hydroxy bile salts, respectively

in pure form (9, 10, 45). Inhibition of lipase by bile salts starts already at low bile salt concentrations and becomes complete when the critical micellar range is reached (Fig. 3). Other anionic detergents, such as dodecyl sulphate and acyl tauroides, also inhibit lipase. This effect cannot therefore be considered specific for bile salt.

Bile salts belong to the so-called mild detergents group which only interact with proteins at specific sites, in contrast to the denaturing detergents of the dodecyl sulphate type which also show so-called co-operative binding on the proteins, leading to conformational changes and denaturation with loss of biological activity (26, 44). Bile salts seem to stabilize the lipase and prevent it from being inactivated on the substrate surface (11).

It seems strange that bile salts, present in the intestinal content of all vertebrates in concentration above the CMC, should inhibit the enzyme present from hydrolyzing the main dietary lipid component the triglyceride. The lipolytic activity of pancreatic juice however is not inhibited by bile salt and it has been found that pancreatic juice contains a co-factor which is able to reactivate lipase in the presence of bile salts (9, 10, 43, 51) (Fig. 4). This co-factor which has been named co-lipase (46) has been purified and is polypeptide of molecular weight $\approx 11,000$ with five disulphide bridges (16, 17, 46). The mechanism of action of co-lipase is not fully understood but evidence indicates that the co-lipase-lipase interaction is entirely physico-chemical, i.e. hydrophobic and/or electrostatic and that the complex formed has modified physico-chemical properties, which makes proper interaction with the substrate possible.

The interaction with the lipase/co-lipase system is not specific for bile salts, it is of interest that the detergents shown to occur in the digestive juice of the Crustacea of the acyl amino acid or acyl dipeptide

type also interact in a similar way with lipase/co-lipase (10).

Sterol ester hydrolase of pancreatic origin. This enzyme which has been shown to be much less specific than the same isozymes, catalyzes the hydrolysis of a wide range of substrates and its activity is greatly stimulated by bile salts (1, 50). It has been shown that this enzyme forms an enzyme bile salt complex involving reversible formation of hexamer which may be the catalytically active form (38). This effect seems to be specific for bile salts and not given by other types of detergents.

Pancreatic phospholipase A_2 . This enzyme is present in the pancreatic juice as zymogen or proenzyme

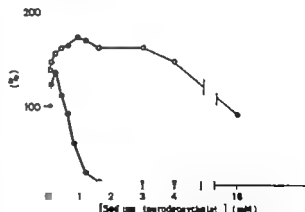


Fig. 4 Lipase activity (V) inhibited by increase in concentration of deoxycholytaurine over the critical micellar concentration (●). Co-lipase prevents the inhibition of lipase (O). The experiment was performed at pH 7.0 in 150 mM NaCl (10).

LIPID CLASSES

NON POLAR

POLAR

 INSOLUBLE
NON SWELLING
AMPHIPHILES

 INSOLUBLE
SWELLING
AMPHIPHILES

 SOLUBLE
AMPHIPHILES

 STEROL ESTERS
VITAMIN ESTERS

 STEROLS
VITAMINS
TRIGLYCERIDE

 FATTY ACIDS - - - - -> SOAP
FATTY ACIDS - - - - -> SOAP
FATTY ACIDS - - - - -> SOAP
MONOGLYCERIDE

PHOSPHOLIPID - - - - -> LYSOPHOSPHOLIPIDS

FATTY ACID - - - - -> SOAP

BILE SALTS

Fig. 5 A physico-chemical classification of dietary lipids and the hydrolytic products formed by the action of pancreatic enzymes thereupon. The broken lines between fatty acid and soap indicate that this conversion most probably is unimportant at the pH values of the intestinal content. The lipids belonging to the class of

soluble amphiphiles form mixed micelles with the bile salts. Fatty acid soaps have higher micellar solubility than the unionized fatty acids even though it is considered that micellar solubilization into bile salt micelles depresses the ionization of the fatty acid.

and is emulsified in the intestinal content by trypsin. It catalyzes the specific hydrolysis of the fatty acid ester bond at the α -position of phosphoglycerides forming free fatty acid and the corresponding lysophosphoglyceride (22).

The phosphoglycerides with long chain fatty acids, as they occur in components of dietary lipid show an absolute requirement for bile salt with the enzyme phospholipase with shorter chain fatty acids such as lecithin with C_{12} -C fatty acid esters, however hydrolyzed by phospholipase without addition of bile salt (43). The importance of bile salt for the hydrolysis of the long chain phospholipids may be seen in the solubilization of the substrate into mixed bile salt-phosphoglyceride micelles. The bile salts then do not act as a co-factor for the enzyme in the normal sense.

Bile salt have been tested as activators of other digestive enzymes but with no positive results (43) with the possible exception of esterolase (3, 52).

The role of bile salts in the solubilization of the products of the biliary digestion

Dietary lipids make up a very heterogeneous group of compounds from a chemical point of view. In a discussion of their behavior in the intestinal lumen they can more conveniently be classified according to their interaction with water and bile salt solutions (12, 36). Lipids can then be defined as molecules containing a hydrophobic hydrocarbon skeleton, which to different extents is substituted by polar (hydrophilic) groups. It is the balance between these two parts which determines the behavior of a lipid in water and bile salt solution. Fig. 5 gives a summary of the classification

of important dietary lipids based on their behavior in water solution. It can be seen that the main components of the dietary fat, triglycerides with long chain fatty acid, are classified as water-insoluble non-swelling amphiphiles. They disperse in bile salt solutions forming emulsions, but their micellar solubility in bile salt solution is very low. Most of the minor components of the dietary lipid such as sterols and sterol esters, fat-soluble vitamins, hydrocarbon etc., show interactions with water and bile salt solutions similar to those of the triglyceride.

Other components of the dietary fat of interest in this respect are the phospholipids. Although this group of lipids for practical purposes is also insoluble in water its members contain enough polar groups to interact with water. Water will penetrate into part of the molecules and cause them to swell and they have been defined as insoluble swelling amphiphiles. These lipids interact with bile salt solutions to form mixed micellar solutions as previously discussed. The effect of bile salts on the dietary lipids is thus an effect of their general detergent properties, forming an emulsion in equilibrium with mixed micellar solution, the partition of the different species between these two physical states being dependent on their non-polar/polar balance. Although most of the lipids of the dietary fat are classified as water-insoluble if these compounds are considered to have a definite but very low water solubility. This third molecular dispersed phase which has been ascribed an important role in the absorption process, is considered to be in equilibrium with the other phases to form a 3-phase emulsion-micellar-water system. The solubility of the long chain fatty acids in water is still low but several orders of magnitude higher than that of the corresponding alkane (?). The ioniza-

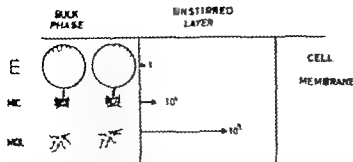


Fig. 11 Importance of the particle size of lipids for their diffusion over the unstirred water layer covering the enterocyte cell membrane. E = emulsion droplets (chiefly triglyceride) diameter = μ , particle size $\approx 4 \times 10^{-5}$ cm; aggregation number $\approx 4 \times 10^3$; MC = micelle diameter ≈ 40 Å, particle weight (non-hydrated)

$\approx 4 \times 10^{-5}$ g., aggregation number ≈ 100 ; MC_2 = molecular dispersed molecular weight ≈ 200 , weight of the single molecule $\approx 4 \times 10^{-25}$ g., aggregation number ≈ 1 . Rate of diffusion related to $1/M$. The thickness of the unstirred layer considered to be within the range of 10^{-2} to 10^{-3} Å (6).

tion of the fatty acids also increases their water solubility (3, 31).

The effect of the lipolytic enzymes in the mucosal content to catalyze the formation of product which are more polar than the substrate and therefore has higher degree of interaction with water and bile salt solutions (Fig. 5). This results in transfer of the product of lipolysis from the oil phase to the mucellar phase to the aqueous phase. The product formed, with the exception of the lysophospholipids which has high water solubility still has very low aqueous molecular solubility. The effect of bile salts in these systems is then to solubilize the polar products formed by lipolysis, namely monoglyceride and fatty acid into mixed micellar solutions. The effect of the mucellar solubilization on the concentration of such compound in the water phase has not been investigated due to lack of suitable methods.

The bile salts also affect the ionization of the fatty acids dissolved into the micelles. The co-operative effects of the solubilization of the hydrocarbon part of the fatty acid to the micellar interior and the presence of neighboring charged bile salt molecules depress the ionization of the fatty acids, their apparent pK_a are increased. This is most probably the mechanism by which bile salts at concentrations above their CMC affect the equilibrium composition of the product mixture catalyzed by pancreatic lipase so that more extensive hydrolysis (relatively more monoglyceride and fatty acid in the equilibrium mixture) is attained at the slightly acid pH values prevailing in the intestinal contents (7).

Transport of lipids over the unstirred layer

It is one of the essential features of the micellar theory of fat absorption that micellar solubilization promotes fat absorption (32). Perfusion experiments in the rat (33) and in man (34) also indicate that this is the case. That triglyceride fat is not hydrolyzed and absorbed to an appreciable extent in the absence of bile has been known for a long time and been explained by the hypothesis that, in the absence of bile, the low

aqueous concentration of fat is not compensated by absorption over a longer period from a larger absorption area (34). The available experimental evidence has nonetheless led to the consideration that fat is not absorbed even in the presence of bile salt as absorbed from the molecular dispersed aqueous solution (40). The explanation of this apparent discrepancy may be found in the importance of the so-called unstirred layer in lipid absorption. The absorptive surface of the intestinal mucosa cell is considered to be covered by a layer of unstirred water through which the molecules to be absorbed has to pass by diffusion (41). It is furthermore considered that the diffusion rate through this unstirred layer is related to the concentration of the lipid in the molecular dispersed phase and that transport of lipid in the absence of bile salt therefore is extremely slow. The bile salt is considered to increase the transport rate over the unstirred layer by presenting the lipid in high concentration in micellar solution to the unstirred layer (42).

The diffusion rate of molecule or particle is directly related to the square root of the molecular weight. If we consider the diffusion rate of an emulsion droplet with diameter of μ , mixed bile salt micelle with hydrated diameter of 40 Å and molecular dispersed fatty acid it can be calculated that their relative diffusion rate will be approximately 10^3 , 10^4 and 10^5 respectively (Fig. 6). These figures are only relative but it can most probably be considered that emulsion particles diffuse so slowly that flux of particles lipid into the unstirred layer is very unimportant in the absorption process. The diffusion rate in the unstirred layer of mixed bile salt micelle is approximately one order of magnitude below that of molecule but on the other hand, as discussed previously, the concentration of lipids in the micellar phase is in many cases several orders of magnitude above that in the molecular dispersed phase. The micellar lipids should thus serve as a pressure head for the molecular dispersed phase (Fig. 7). Stated in another way, the function of the micelles is to overcome the resistance of the unstirred layer.

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TRIGLYCERIDE

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MONOGLYCERIDE

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↓
FATTY ACID -----> SOAP

BILE SALTS

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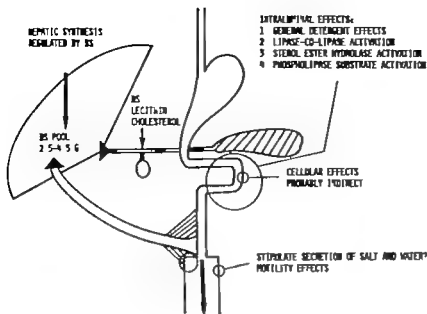


Fig. 8 Summary of the effects of the bile salts in the gastrointestinal tract. Bile salts also are of importance for the solubilization of lecithin and cholesterol of the bile, but this function is not considered in this review.

et al. (70) made the interesting observation that if the upper small intestine such secretion is abolished by the simultaneous presence of lecithin. The inhibitory effect of lecithin may be due to its property of decreasing the actual concentration of bile salts in molecular form. The dihydroxy bile salts also cause water secretion from the intestine; the secreted fluid is mainly sodium chloride solution and the effect is certainly not an osmotic effect. The physiological importance and the mechanism of this effect is unknown, but it has been suggested that physiological function of bile salts in the colon is to prevent undue dehydration of feces (19). When bile salt spills over into the large intestine due to incomplete absorption as seen after ileal resection and diseased states of the distal ileum, bile-acid-induced inhibition and secretion of water explains the diarrhea seen in these conditions (35). Bile salts also have less well defined effects on gastric emptying (39), intestinal motility and the secretion of intestinal hormones (40).

TO WHAT EXTENT ARE THE FUNCTIONS OF BILE SALTS UNIQUE?

As noted, bile salts are detergent and the question is whether all the functions of the bile salts in the intestinal tract can be considered as general detergent effects or to what extent they can be said to be specific or unique for the bile salt. The bile salts can be said to have some special detergent properties. In general, they form smaller micelles than most other detergents and have a relatively high CMC, they show no cooperative binding to proteins (44) and therefore have no denaturing effects. They have a high capacity—in comparison with other detergents to dissolve water-insoluble, swelling amphiphiles such as monoglycerides and fatty acids into mixed micellar solutions—and low

capacity to solubilize non-polar lipid. The inclusion into the bile salt micelle of monoglyceride and long chain fatty acids, on the other hand, greatly increases the cosolubilization of non-polar lipids. These properties of the bile salt micelle are of particular importance for intestinal absorption, as they make lipolysis of paramount importance for the absorption of both the triglyceride fat and other lipids of dietary origin dissolved in the triglyceride. The products of lipolysis, the monoglycerides and fatty acids, may be said in this way to provide solvent vehicle for other non-polar lipids, the absorption of which is dependent on the transport of fatty acids and monoglyceride in the micellar phase. Non-polar lipids such as hydrocarbons, fat-soluble vitamins, etc. when fed alone are scarcely absorbed at all from the intestinal tract. When fed together with triglyceride fat, appreciable quantities are absorbed due to their much greater solubility of this compound in the mixed bile salt micelles containing the hydrolytic products of the triglycerides. The extent of absorption of non-polar lipid therefore due to the special detergent properties of the bile salts, becomes dependent on its rate of absorption in relation to the triglyceride hydrolytic product.

In vitro as well as in vivo experiments have definitely shown that fatty acids can be absorbed when presented to the intestine in the form of micellar solution in non-bile salt detergent (11, 67).

What, then, are the other functions of bile salt? As mentioned in the introduction, detergents other than those containing the steroid nucleus have been found in the digestive juice of some invertebrates, notably the Crustacea. This type of detergent, which is of the fatty acyl amino acid type, has been found to replace bile salts in the interaction with lipase/co-lipase, but their ability to do so in other functions has not been investigated and no other detergent has so far been found to replace the bile salts in their function associated

with sterol ester hydrolase or phospholipase A_2 . The bile salt therefore may be considered specific in these two functions, even though they are reflections of the detergent properties of the bile salt.

From a clinical point of view it would be of interest to have a substance that interfered with the functions of bile salt in the intestinal content. If a controlled malabsorption of fat could be established (this could be of importance for the treatment of certain abnormalities of lipid metabolism). The only type of compound so far introduced for such purposes is insoluble cationic exchangers such as cholestyramine and DEAE-Sephadex, which bind bile salts resulting in an increased fecal excretion and enhanced synthesis of bile acids from cholesterol (21). When given in adequate amount they also are able to produce a micellar phase deficit, thereby leading to malabsorption. As bile salts are important for the function of the lipolytic enzymes in the intestinal tract, another possibility would be to find substances which interfere with the function of these enzymes. *In vitro* the most potent inhibitors of pancreatic lipase are found among the cationic detergents exemplified by hexadecyl-(acetyl)-trimethyl ammonium bromide. This compound inhibits lipase in concentration low enough to be practical to produce *in vivo*. Its general toxicity probably makes it useless for these purposes.

One remarkable feature of the metabolism of the bile salts is the enterohepatic which is of paramount importance for their physiological function. This property involves an enterohepatic circulation. The importance of the enterohepatic circulation which is characterized by a distal active reabsorption of the bile salts is to maintain a high concentration of bile salts in the upper small intestine where absorption of the hydrolytic products of the nutrients takes place, and to ensure the daily circulation of bile salts in amounts greatly in excess of the pool size and the synthetic capacity of the liver. When the distal absorption of bile salts is not functioning, the bile salt pool may be washed out in a single passage of the intestinal tract, resulting in a micellar phase deficit. Some of the requirements for an active distal absorption related to the bile salt structure have been studied (41) but no substitute for the bile salt skeleton has been searched for.

CONCLUSION

The secretion of bile salts or analogues into the intestinal tract is a universal feature of all vertebrates, a fact that indicates a general importance of these substances for the function of the intestinal tract. Our present knowledge of the function of bile salts in the digestive tract can be summarized in the following points (Fig. 8).

1. A general detergent function: dispersing and solubilizing in the intestinal content dietary fats and their hydrolytic products in micellar form, thereby facilitating their uptake into the intestinal cell membrane.
2. Effects on the function of the lipolytic enzymes secreted in the pancreatic juice. (a) An interaction

with pancreatic lipase and a polypeptide co-factor for this enzyme named co-lipase. (b) An interaction with sterol ester hydrolase to form a catalytically active enzyme/bile salt aggregate. (c) To disperse water-insoluble long chain phosphoglycerides in mixed micellar state so that they can serve as substrate for phospholipase A_2 . (d) To activate enterokinase?

3. To stimulate the secretion of water and salt from the small and large intestine.

4. To affect the motility of the intestine and the secretion of intestinal hormones.

All recognized functions of bile salts for fat absorption take place in the intestinal content outside the body. Effects of bile salt claimed to occur intracellularly in the enterocyte are most probably secondary to the intraluminal effects. The question has been considered as to what extent the functions of bile salts are general detergent effects or to what extent they can be considered unique.

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BLOOD PRESSURE AND BLOOD LIPIDS IN MEMBERS OF FAMILIES WITH A HEAVY AGGREGATION OF ESSENTIAL HYPERTENSION

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Abstract Eighty-four persons between 17 and 48 years of age who were siblings or children of hospitalized patients with essential hypertension and whose families had additional cases of high BP and/or manifestations, have been investigated. BP, blood lipids and some other parameters were measured. As control group served 81 age-matched persons, randomly selected from a general health survey. None of these controls had hypertensive disease in their families. Significantly higher BP was found in patients over the age of 33 with family history of hypertension than in the control group. No difference below this age could be found. No significant differences in blood lipid levels were found.

High blood pressure and its complications have long been observed to occur in certain families. As early as in 1761 Morgagni described five cases of familial apoplexy (11). From then on there have been several reports of single families and studies of family histories (2, 5, 12, 18) hypertensive patients being the pilot cases. Most of these have indicated a familial factor in hypertension; this factor has usually been considered to be mediated by a dominant gene (2, 13, 18). Others have been less definite about the role of heredity (19). Family histories taken from patients have often been suggested to stress the occurrence of the disease in relatives and may therefore present a false picture. In addition histories most often record arterial disease the cause of which is not always hypertension.

The criticisms mentioned were first put forward in 1954 by Hamilton et al. (8) who reported a study of BP measurements in a population sample: in relatives of hypertensives and in relatives of controls. BP was shown to increase with age in all three samples though in the second group the recordings were skewed towards higher pressures

than in the other groups in which they were roughly on the same level. The authors concluded that BP is multifactorially determined and at least partly inherited. The nature of these factors has been discussed by several authors in terms of heredity and environment (4, 7, 10, 14, 19); most papers favouring heredity as being the principal contribution (4, 10, 14). Strong support has recently been lent to this theory by investigations in early childhood which revealed clustering of raised BP within families (20).

The present study is an investigation of the BP level, the blood lipid levels and certain other parameters of members of families apparently susceptible to essential hypertension and its complications. We have found no earlier study in which families at high risk in this respect have been subjected to an investigation of this kind. The aim from a practical point of view was to try to establish the age above which it would appear mandatory to supervise these individuals closely in order to detect the possible appearance of hypertension.

MATERIAL

The names were collected of all patients admitted to the Medical Department of the University Hospital in Uppsala over the last three years who were registered as suffering from arterial hypertension shown to be of essential type in the cumulative diagnosis index. Their medical histories were studied for evidence of familial hypertension. Patients, whose case histories revealed one or more family members who had either raised BP detected before the age of 55 or had a stroke or developed cardiac insufficiency not due to organic valvular disease before their 70th birthday were questioned concerning siblings and children. Thus myocardial infarctions or hypertension in connection with

Diastolic BP mmHg



Fig 1 Age versus RSDP in males. ■=siblings or children in families with at least two known members with hypertensive disease <65–70 years. □=controls from general health survey who denied a family history of hypertension and/or cardiovascular disease before the age of 65

renal insufficiency in the history were not accepted as evidence of familial hypertensive disease and those patients were excluded from the study.

All the relatives (84 persons: 39 men and 45 women) aged between 17 and 49) thus collected were invited to participate in the investigation and to undergo an out-patient examination. A thorough medical history was obtained from each. No case of renal insufficiency was detected by this screening.

A control group of 61 persons, 77 men and 34 women, were drawn consecutively from a group attending a general health survey. Each control subject was 1 with regard to age with one of the patient (±3 years at the time of examination). The control group came from the same community as the patient group and the known presence of family members with hypertension or cardiovascular disease (as defined above) was excluded by a detailed medical history. A sufficient number of controls were collected to allow statistical analysis. To ensure sufficient numbers for blood lipid estimations, nine persons were added to the control group who did not participate in the BP study

Table I Age distribution

	Total	Age group			
		<33 y		≥33 y	
		Men	Women	Men	Women
Familial group					
Mean	31.9	24.0	25.8	38.6	40.3
N	84	20	4	19	21
Control group					
Mean	31.1	24.6	26.4	38.8	39.4
N	61	12	13	9	19

Diastolic BP mmHg

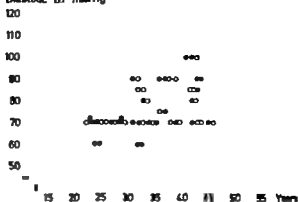


Fig 2 Age versus RSDP in females. ●=siblings or children in families with at least two known members with hypertensive disease <65–70 years. ○=controls from general health survey who denied family history of hypertension and/or cardiovascular disease before the age of 65

though it had been confirmed that they had no family history of hypertension.

METHODS

BP was recorded indirectly using a 13 cm cuff on the right arm connected to a mercury column. Two measurements were made for each subject: 1) directly on arrival (supine) and 2) after 10 min rest (supine). The disappearance of the fifth sound of Korotkoff was taken as the diastolic BP level. Height and weight were measured. Physical examination included auscultation of the heart and palpation of the femoral arteries. Blood was drawn for cholesterol, triglycerides, creatinine as well as Hct and ESR. Urine was analysed for protein, glucose and cells. An ECG was recorded from which the pulse rate was read except in 16 controls for whom ECG was lacking due to various reasons. In those cases the pulse rate was registered by digital palpation of the radial artery. Each examination was carried out by one of the authors and was performed in the same examination room with the same instruments. As a rule the examination took place in the morning with the subject

Table II Means of resting supine diastolic pressure (RSDP)

	Age group		Total
	<33 y	≥33 y	
<i>Familial group</i>			
Men	71.0	87.4	79
Women	73.3	83.7	79.5
Men+women	72.2	86.5	79.4
<i>Control group</i>			
Men	67.5	75.6	71.5
Women	72.3	77.9	75.1
Men+women	69.9	76.7	73.3

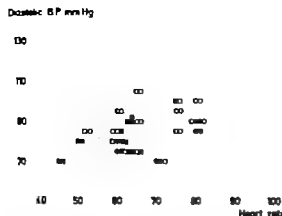


Fig. 3 Resting pulse rate versus RSDP in members of families with heavy aggregation of essential hypertension. ■—males <33 years □—males ≥33 years ●—females <33 years ○—females ≥33 years.

fasting. Exceptions were most of the controls, who were examined later in the day and were not fasting. Cholesterol and triglycerides of the latter were checked on a later day in the morning and in fasting state.

From the outset the clinical material was divided on the basis of age and family history. In addition each group was arbitrarily divided into two: those between 17 and 32 years of age and those between 33 and 49. This division produced similarly sized groups (Table 1). The resulting groups served as cells in the unweighted analysis of variance. Testing of hypotheses was done according to *F*-test, and the level of significance was 5%. Additional analyses were made using contrasts (the level of significance corrected by Fisher method) and Hotelling T^2 -test.

RESULTS

Blood pressure Resting supine diastolic pressure (RSDP) was used in the statistical analysis. The

Table III Means of resting supine systolic pressure (RSSP)

	Age group		Total
	<33 y	≥33 y	
<i>Familial group</i>			
Men	132.0	140.5	136.3
Women	125.6	148.1	136.9
Men + women	128.8	144.3	136.6
<i>Control group</i>			
Men	131.1	132.8	132.0
Women	125.3	132.4	128.9
Men + women	128.2	132.6	130.4

mean RSDP was found to be significantly higher in the groups with positive family history than in the control groups ($p < 0.05$) (Table II Figs 1–3). No significant difference could be attributed to sex in the different groups. BP as expected was found to be higher in the older groups (controls and familial groups taken together) ($p < 0.05$). When variance was analysed a nearly significantly higher BP ($F = 3.7$) was found in the older groups with a positive family history than in the corresponding control groups. This was confirmed by additional analyses using contrasts. No such difference could however be demonstrated between the younger groups.

The means of the resting supine systolic pressure (RSSP) showed the same pattern among the groups as the RSDP (Table III). However the variance was greater within the groups.

The distribution of the systolic and diastolic BP in the total material is demonstrated in Figs 4 (younger groups) and 5 (older groups). By drawing a line cutting the abscissa between 90 and 95 mmHg diastolic pressure in Fig. 5 for instance, the difference between the familial and the control groups is easily demonstrable. To the right of this line 1 familial case is situated (30% of the older familial group) as compared to a single case of the control group (4% of the older control group). By drawing a line cutting the ordinate horizontally between 150 and 155 mmHg systolic pressure an equivalent situation is found concerning the systolic pressures above this line (3 and 11% respectively). The differences between the casual supine diastolic pressure (CSDP) and the RSDP (Δ CSDP–RSDP) on the one hand, and the casual supine systolic pressure (CSSP) and the RSSP (Δ CSSP–RSSP) on the other were calculated. Δ CSDP–RSDP was 4.4 mmHg in the familial groups and 1.3 mmHg in the controls. The corresponding figures for Δ CSSP–RSSP were 5.1 and 3.0 mmHg respectively. These numerical differences are not statistically significant.

The resting pulse rate was surprisingly lower in the familial groups than in the controls and the difference was statistically significant ($p < 0.005$) (Table IV). The pulse rate was significantly higher in women than in men irrespective of age ($p < 0.005$). The pulse rates obtained by digital palpation did not differ significantly from those obtained from ECGs. Plotting the RSDP versus the resting pulse rate in the familial groups gave a pat-

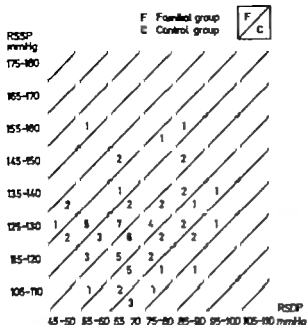


Fig 4 Distribution of RSP and RSDP in younger age groups

tern with no individuals of the younger age groups situated in the low pulse-high pressure quadrant (Fig. 3). Calculation of the correlation coefficient in the different age and sex groups showed a significant positive correlation between RSDP and pulse rate in the familial group of young men only ($r=0.63$ $p<0.005$). The same was for RSP in relation to resting pulse rate in familial group of young females ($r=0.55$ $p<0.01$). No such correlations were found in controls. The correlation between RSDP and resting pulse rate was not significantly changed on successive testing of still younger (and smaller) groups of males belonging to the families. However below the age of 23 the number of individuals was too small to reach significance.

Blood lipids The means of the serum triglycerides and serum cholesterol were higher in the older than in the younger groups and the difference was statistically significant ($p<0.05$) (Table V). There was no difference in serum triglyceride level between the familial and the control groups. However such a difference was demonstrable concerning serum cholesterol which was significantly higher in the familial groups ($p<0.05$).

Anthropometric data Length and weight did not differ between the familial and the control groups ($p>0.25$) (Table VI).

Table IV Means of resting pulse rate

	Age group		Total
	<33 y	≥33 y	
<i>Familial group</i>			
Men	64.5	66.1	65.4
Women	71.5	70.4	71.0
<i>Control group</i>			
Men	65.9	71.4	68.0
Women	76.9	75.1	76.0

DISCUSSION

The finding of a higher BP in the familial groups than in controls is in accordance with earlier investigations of family members of patients with assured hypertension regardless of its cause (4, 8, 17) and with similar studies in which the hypertensive index cases, as in this study had first been investigated to rule out secondary hypertension (7, 14).

The rise of BP with age as demonstrated in this work is common medical knowledge and is

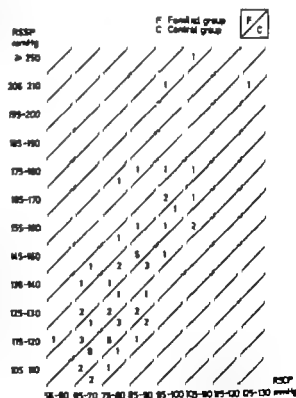


Fig 5 Distribution of RSP and RSDP in older age groups.

Table V Means of serum triglycerides and serum cholesterol

	Age group		Total
	<33 y	≥33 y	
<i>Serum triglycerides (mmol/l)</i>			
Familial group			
Men	1.30	1.45	1.38
Women	1.14	1.28	1.21
Men+women	1.22	1.37	1.29
Control group			
Men	1.05	1.57	1.28
Women	0.94	1.35	1.14
Men+women	0.99	1.43	1.11
<i>Serum cholesterol (mg/100 ml)</i>			
Familial group			
Men	222.4	238.3	230.3
Women	101.1	41.4	225.8
Men+women	216.3	239.8	228.1
Control group			
Men	188.6	223.9	206.3
Women	184.4	42.6	13.5
Men+women	186.5	233.3	209.9

well documented in the literature e.g. in the large population study by Hamilton et al (8).

The resemblance of BP within families in different age groups in young people and small children, has been demonstrated before (8-10). Smirk (17) found in a controlled study of the BP level of 319 first degree relatives of propositi with "substantial hypertension" that the casual and basal BP are higher in the relatives especially above the age of 40. However Smirk was con-

cerned with hypertension regardless of its cause he accepted any first degree relative and did not, as in our study pick out those with additional cases of hypertensive disease in the family. The finding of a cut-off point of age above which there is a considerable increase of the BP level in members of families with a heavy aggregation of essential hypertension has its practical implications in the important field of preventive medicine. We have found no earlier study of this specific kind in the literature. Thus on the basis of this investigation it seems reasonable to start screening of members of such high risk families at the age of 30-35.

Smirk found in an early study (16) that the difference between the casual and basal BP was greater among hypertensive individuals than among normal subjects. However he did not find this to be so in his study of relatives of hypertensives referred to above (17). The difference in our study between casual and resting BP is numerically greater in the familial groups than in controls but not sufficiently for statistical significance.

The somewhat unexpected finding of lower resting pulse rates in the familial groups compared to controls is probably attributable to the fact that all familial cases were examined in the early morning, and this was the case for only a minor part of the controls, the majority of whom were seen in the afternoon. Richardson et al found in 1964 that the pulse rate rises during the day both in normal and hypertensive subjects (15). Our finding of higher pulse rates in women than in men is in accordance with earlier investigations (3).

The finding of a significant positive correlation between BP and pulse rate in the younger familial groups but in no other group is consistent with the concept of essential hypertension starting as a high output pressure with normal peripheral resistance later merging into a normal or low output pressure with raised peripheral resistance (9).

Our finding of higher blood lipid levels in the older than in the younger groups is in accordance with earlier investigations concerning both serum triglycerides and serum cholesterol (6). The reason for the unexpected finding of higher serum cholesterol in the familial groups than in controls cannot be sought in the variation of body fat mass (1) because length and weight are on the same level. In addition such a cause would reasonably affect

Table VI. Anthropometric data

	Age group		Total
	<33 y	≥33 y	
<i>Body weight (kg)</i>			
Familial group			
Men	74.42	76.35	75.41
Women	63.13	63.65	63.37
Control group			
Men	72.29	77.85	74.70
Women	59.00	64.58	61.39
<i>Body length (cm)</i>			
Familial group			
Men	179.8	175.5	177.6
Women	165.7	161.3	164.1
Control group			
Men	180.1	179.3	179.8
Women	168.7	165.0	166.9

the serum triglycerides to a greater extent (1) As no adequate methodological control was practised in the laboratory consulted at the start of the study and the bulk of the familial cases were examined before the mass of controls it cannot be excluded that there may have been some methodological drift during the course of the investigation.

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DOPAMINE β -HYDROXYLASE IN HYPERTENSION

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Abstract Dopamine- β -hydroxylase (DBH), the enzyme responsible for the biosynthesis of noradrenaline from dopamine, has been assayed in the blood plasma of 53 hypertensive patients. The results were compared with those from a control group of 93 adults. The plasma DBH activity varied within a wide range in both hypertensives and controls. There was no difference between the average DBH activity nor any significant relation between the resting recumbent systolic or diastolic BP and the plasma DBH activity. In a subsample of this material consisting of 11 patients and controls matched for age and sex, the DBH activity was studied at 3-hour intervals during 12 hours in an attempt to estimate the variation of each individual. No significant differences were found between the two groups.

The role of the sympathetic nervous system in the pathogenesis of primary hypertension is not known. Recently some reports on the catecholamine level in serum from hypertensive patients have been published. De Quattro and Chan (7) described an increased level in patients with primary hypertension. Furthermore, Loois et al. (4) found a highly significant ($p < 0.001$) relation between the resting recumbent BP and the plasma level of noradrenaline.

The enzyme dopamine- β -hydroxylase (DBH), which is responsible for the synthesis of noradrenaline from dopamine, is released together with noradrenaline from the sympathetic nerve terminals and can be detected in the blood (11). The blood level of DBH may then be a measure of the activity of the sympathetic nervous system (11). If this system is in hyperactivity in the state of hypertension, it might be reflected by an elevated level of DBH in the blood circulation. In the present study we have investigated the level of plasma DBH activity in patients with primary hypertension, the relationship between the BP level and DBH, and finally the daytime variability.

MATERIAL A

Seventy female and 46 male patients with hypertension were studied (Table I). The mean age was 45.7 ± 14 years (range 17-71). They were selected on a first available basis and mainly collected during a time span of 6 months. The patients had primary hypertension, except three who had renovascular causes of their hypertension. They had never received any antihypertensive drug.

Plasma DBH was sampled in the morning with the patients fasting. Immediately before the plasma sampling BP was measured by the auscultatory method after 10 min rest. This material was evaluated with regard to the level of plasma DBH and whether any relationship existed between DBH and resting BP.

The control group consisted of 93 apparently healthy adults of the hospital staff and blood donors of both sexes with the same age distribution as the hypertensives.

MATERIAL B

Eleven patients (Table I) with primary hypertension were studied. The first DBH was taken under similar conditions to those in material A. The other specimens were taken at 3-hour intervals over 12 hours (a total of 5 specimens from each patient). The patients were on regular daytime hospital activities. None had antihypertensive therapy. In the calculations the lowest value of the five specimens was determined as 100 (arbitrary units). The different values were then recalculated and could be added together for usual statistical analysis.

The control group was obtained from the Departments of Medicine and Surgery, University Hospital, Uppsala. The controls were matched with regard to age and sex and the only conditions for acceptance were normotension and lack of medication. The group variability was estimated as in material B.

METHOD

Venous blood was collected into heparinized plastic tubes. Within half an hour the blood was centrifuged and the plasma separated and stored at -20°C until assayed.

Table I Data on the patients

	Material A	Material B
No. of pts.	53 (♂/♀ 46/7)	11 (♂/♀ 7/4)
Age (y)	45.7 ± 1.4	44 ± 3.9
Heart volume (ml/m ² BSA)	423 ± 2.3 (300-750)	438 ± 3.9 (300-750)
Serum creatinine (mg/100 ml)	1.0 ± 0.06 (0.7-1.8)	0.9 ± 0.06 (0.7-1.2)
No. of pts.		
LVH* (ECG)	5	4
FF*		
0-1	20	5
1	14	
	19	4

ECG criteria according to Goldman. Keith-Wagener classification.

Table II Results of the determination of DBH activity

	No. of cases	Mean	Range	Median	Quantile	
					Lower	Upper
Hypertensives (A)	53	214 ± 18	5-569	186	119	287
Control	93	225 ± 16	0-715	193	102	319

DBH was determined enzymatically according to the method of Molinoff et al. (6) as modified by Weinsblum et al. (13). β -phenylethylamine was used as the substrate and the pH of DBH was kept at 5.2, the optimal pH for this enzyme. The incubation mixture consisted of 200 μ l blood plasma diluted 1:50 with a 5x M solution of CuSO₄ in distilled water, 25 μ l 8 M ascorbic acid, pH 6, 25 μ l 0.5 M sodium formate, pH 6, 20 μ l 0.006 M purpyrine, 1500 U catalase, 10 μ l distilled water, 10 μ l 0.03 M β -phenylethylamine and 10 μ l 1.0 M sodium acetate buffer, pH 4.9.

After 20 min incubation at 37°C the DBH reaction was stopped and the enzymatic determination of the β -phenylethanolamine formed was started by addition to each tube of 100 μ l of a fresh mixture of 10 μ l purified PNMT (phenylethanolamine-N-methyltransferase), 10 μ l S-adenosylmethionine, methyl-¹⁴C (1 nmol) and 80 μ l 1.0 M Tris buffer, pH 8.6. After 30 min incubation the reaction was stopped by addition of 0.5 ml 0.5 M borate buffer, pH 10. The N-methyl-¹⁴C phenylethanolamine formed was extracted by vigorous shaking into 6 ml toluene containing 3% homonyl alcohol.

Table III Variability within material B and controls (arbitrary units)

	No. of cases	Mean	Range	S.E.M.
Hypertensives (B)	11	123	ns (100)-240	4.0
Controls	11	115	ns (100)-157	3.9

ns=not significant.

After centrifugation 4 ml of the organic layer were transferred to counting vials containing 10 ml scintillation liquid and the radioactivity was determined. Blanks consisting of diluted plasma heated to 95°C for 5 min and internal standards (100 ng phenylethanolamine hydrochloride) were run through the whole reaction procedure. The internal standards were used for the calculation of the DBH activity expressed as nmol of β -phenylethanolamine formed from 1 ml plasma during 20 min incubation. The DBH activity was determined in triplicates and the mean value is reported. A plasma sample was used as an external standard in order to make the results from different assays comparable.

RESULTS

In the control group as well as in the hypertensives the plasma DBH activity varied within a very wide range. The activity of one patient was less than 10 nmol/ml/20 min. Six of the controls had no detectable DBH activity. (Among 91 hypertensives so far we have found none without measurable DBH activity compared to 6 of 93 controls.) The average DBH activity in material A was 214 ± 18 and in the control group 225 ± 16 nmol/ml/20 min. The difference between the means was not significant (Table II).

The difference in variability within material B and within the control group did not reach significance (Table III). Finally no significant relation

was found in material A between plasma DBH activity and the resting recumbent systolic BP ($r=-0.156$, $y=178x-0.020$) or the diastolic BP ($r=0.052$, $y=111x+0.004$)

DISCUSSION

Observations in several laboratories have shown that there is a great individual variation of the plasma DBH activity (2, 8, 13, 14). Alterations in DBH activity induced by disease may therefore be lost in this wide range of activity. The variation of the plasma DBH seems not to be related to differences in sympathetic nerve activity since there are apparently healthy persons with no detectable DBH activity in their blood. In a recent study of the plasma DBH in monozygotic and dizygotic twin pairs it was observed that the level of the plasma DBH is genetically controlled (9). The same conclusion was independently made by Weinsztein et al. (13) from the results of a sibling study. Other factors than the sympathetic nerve activity may accordingly determine the level of the plasma DBH activity. This hypothesis does not preclude that the sympathetic nerves and the adrenal medulla are the main sources of the plasma DBH. In order to unmask the influence of the wide individual variation it would be preferable to use healthy siblings as controls in studies of the DBH activity in disorders (5).

In a preliminary study (14) we reported an elevated DBH activity in hypertension. This result is not verified by the present extended study and was apparently due to an insufficient material. Horwitz et al. (3) were also unable to observe any changes in serum DBH activity in a study of hypertensives. These observations do not necessarily mean that plasma DBH activity does not rise in hypertension in the individual but they indicate that the elevation is not large enough to be perceptible in the wide individual variation. An observation that indicates that this really may be the fact is the lack of subjects without detectable DBH activity among the hypertensive material whereas six persons were without plasma DBH activity in the control material.

The conclusion drawn from the present study is that there is no difference in plasma DBH activity in a random sample of hypertensives compared to controls. The reason for this negative finding may be that other factors than the activity of the

sympathetic nervous system dominate the regulation of the plasma DBH level. A study of hypertensive patients with their healthy siblings as controls can perhaps elucidate whether the plasma DBH like plasma noradrenaline (1, 4, 7) is elevated in hypertension.

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THE EFFECT OF POTASSIUM CHLORIDE AND SPIRONOLACTONE ON THIAZIDE INDUCED POTASSIUM DEPLETION IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Abstract. Total exchangeable potassium (K_E), red blood cell potassium (K_{RBC}), serum potassium (K_S) and blood pressure (BP) have been determined in 34 patients with essential hypertension after not less than 4 weeks therapy with hydrochlorothiazide 75 mg daily. K_E was significantly lower than the levels found in untreated patients with essential hypertension, determined by the same method and in the same laboratory. K_{RBC} and K_S were also significantly lower than the values in normal individuals. The patients were then divided at random into two groups, one group receiving potassium chloride supplement of 60 mEq/d, the other spironolactone supplement of 100 mg/d. After 5 weeks of combined therapy the above parameters were again determined. Both groups presented a significant rise of K_E , K_{RBC} and K_S . The rise of K_E in the spironolactone group was significantly higher than that found in the potassium chloride group, but the increase of K_E and K_{RBC} in the two groups did not differ significantly. Spironolactone caused a significant reduction in systolic as well as diastolic BP, while potassium supplement did not influence the BP.

With a few exceptions (1-14) numerous studies have shown that continuous diuretic therapy will produce hypokalaemia and a reduction in total body potassium (2, 11, 12, 17, 20). This is supported by the finding in muscular tissue of reduced potassium levels following diuretic therapy (3).

To counteract this potassium deficiency supplements of potassium chloride, spironolactone or another potassium-sparing diuretic may be added. Such therapy will cause the serum potassium to rise, but it remains to be clarified whether this is accompanied by an increase in the total body

potassium. Results of studies on this problem are contradictory.

The object of the present study was to examine the effect of potassium chloride and spironolactone supplements on total exchangeable potassium (K_E), red blood cell potassium (K_{RBC}) and serum potassium (K_S) in patients with arterial hypertension on long-term hydrochlorothiazide therapy.

MATERIAL AND METHODS

Studies were performed on 34 patients, 23 males and 9 females, with essential hypertension of slight to medium severity. All patients had been investigated to exclude known causes of hypertension, the programme including determination of serum electrolytes, urinary excretion of catecholamines, angiography and isotope renography. None of the patients had present or previous cardiac failure, and in all of them normal renal function was found assessed on the basis of serum creatinine and creatinine clearance. Twenty of the patients had received thiazide for more than one year, on an average 4 years prior to the study. The remaining patients had been treated for only a few months or had started treatment at the commencement of the study.

The patients were given 75 mg hydrochlorothiazide three times daily for at least 4 weeks. No other antihypertensive therapy was given, and any previous supplement of potassium or potassium-sparing diuretics was withdrawn. K_E , K_{RBC} and K_S were then determined. BP was measured on the same day. All investigations were made in the Outpatient Clinic while the patients were fasting. No other dietary restrictions were made, and the patients were instructed not to change their normal diet. The aim of the study was explained to the patients and all agreed to participate.

Following the determination of the initial values, the patients were divided at random into two groups. One group received supplement of 20 mEq potassium chloride three times daily and the other 25 mg spironolactone four times daily. After 5 weeks of combined

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Table I K_{B} in thiazide-treated and untreated (6) hypertensive men and women

	Thiazide treated	Un-treated	p
Men			
No.	25	1	
Mean age (y.)	50	49	
K_{B} (mEq/m ² BSA)			
Median value	1.41	1.628	<0.05
Interquartile range	1.306-1.523	1.366-1.718	
Women			
No.	9	22	
Mean age (y.)	48	54	
K_{B} (mEq/m ² BSA)			
Median value	1.128	1.387	<0.01
Interquartile range	0.978-1.156	1.168-1.476	

therapy the above parameters were again determined. Spiroolactone (Aldactone®) was placed at our disposal by G. D. Searle Ltd.

K_{B} was determined according to Christensen and Gahkro (6). Approximately 75 μC ^{42}K were injected i.v. at 8 a.m. After 24 hours equilibration the specific activity was determined in a 1-hour spot urine sample obtained between the 24th and 25th hour after the injection. ^{42}K was counted in liquid sample Geiger-Müller counter III was then calculated according to the ordinary isotope dilution principle.

K_{BAC} was determined as described in another paper (3). This method is based on determination of whole-body potassium (K_{B}), K_{BAC} and haematocrit (H). K_{BAC} is then be

$$K_{\text{BAC}} = \frac{100}{H} (K_{\text{B}} - K_{\text{B}}) + K_{\text{B}}$$

expressed in mEq/l packed red cells.

1.45 controls, the normal range of K_{BAC} expressed as the 2.5 and 97.5% percentiles, was 94.0-105.7 mEq/l

(median value 98.4 \pm 45). The results found in males and females were identical. K_{B} in the same 45 controls, expressed as the 2.5 and 97.5% percentiles was 3.6-4.8 mEq/l (median value 4.3).

BP was measured with an armlet and a mercury manometer at 8 a.m. after at least 10 min in supine position. The values stated are the averages of three consecutive measurements.

One patient had to be excluded after 4 weeks combined therapy with thiazide and piroxolactone because of menometrorrhagia.

Statistical calculation were made by employing the Student's *t*-test for paired observations and the Mann-Whitney rank-sum test for unpaired data. When unpaired data were compared they were expressed by median value and interquartile range (central 50% range). In order to evaluate the reproducibility of the method for measuring K_{B} two renal determinations were made in 10 patients at intervals of 1 week, the S.D. of these being 163 mEq.

RESULTS

After at least 4 weeks therapy with hydrochlorothiazide alone (Table I) the total K_{B} in males and females indicated by median value and interquartile range was 1.412 mEq/m² BSA (1.306-1.523) and 1.128 mEq/m² BSA (0.978-1.156) respectively. These values are significantly lower than those found by Christensen and Gahkro (6) in males and females with untreated hypertension: the age distribution of their patients being similar to that of the present series. K_{BAC} levels were also lower than those found in normal subjects: median value 97.9 mEq/l (interquartile range 90.4-94.4) ($p < 0.01$). As stated above the normal range of K_{BAC} was 94.0-105.7 mEq/l; the median value being 98.4 mEq/l. K_{B} was also low: median value 3.2 mEq/l (interquartile range 2.9-3.4).

Table II Change in K_{B} , K_{BAC} , K_{B} and BP during combined treatment with hydrochlorothiazide and potassium chloride for 5 weeks in 17 patients

	K_{B} (mEq/m ² BSA)	K_{BAC} (mEq/l packed cell)	K_{B} (mEq/l)	BP (mmHg)	
				Systolic	Diastolic
Thiazide (75 mg/d)					
Mean	1.364	92.6	3.2	153	97
S.D.	± 217	± 2.4	± 0.3	± 19	± 7
Thiazide + potassium chloride (60 mEq/d)					
Mean change	+153	+2.6	+0.35	+4	0
S.D. of change	± 148	± 3.0	± 0.21	± 13	± 10
<i>t</i>	4.2575	3.6032	6.6475	1.4543	0.0733
<i>p</i>	<0.001	<0.005	<0.001	n.s.	n.s.

Table III Changes in K_{e} , K_{acc} , A and BP during combined treatment with hydrochlorothiazide and spironolactone for 5 weeks in 16 patients

	K_e (mEq/m ² BSA)	K_{acc} (mEq/l packed cells)	K_a (mEq/l)	BP (mmHg)	
				Systolic	Diastolic
Thiazide (75 mg/d)					
Mean	13.0	92.6	3.1	148	93
S.D.	±207	±3.3	±0.5	±14	±7
Thiazide + spironolactone (100 mg/d)					
Mean change	+ 81	+ 7	+ 0.88	- 18	- 7
S.D. of change	±104	±3.3	±0.33	± 9.6	± 8.4
r	0.1080	0.3066	0.5654	0.7390	0.4999
p	< 0.01	< 0.005	< 0.001	< 0.001	< 0.005

After 5 weeks combined therapy with 75 mg hydrochlorothiazide and 60 mEq potassium chloride daily K_{e} increased significantly on an average 11% ($p < 0.001$) (Table II). Also K_{acc} rose significantly by an average of ~ 6 mEq/l ($p < 0.005$) and K_{a} increased by 0.35 mEq/l ($p < 0.001$). BP was not influenced by the potassium supplement.

Patients who received spironolactone supplement 100 mg daily presented a significant increase in K_{e} on an average 6% ($p < 0.005$) (Table III). K_{acc} increased on an average by ~ 7 mEq/l ($p < 0.005$). There was a considerable increase in K_{a} 0.88 mEq/l on an average ($p < 0.001$). Both systolic and diastolic BP were significantly reduced 18 ($p < 0.001$) and 7 mmHg ($p < 0.005$) respectively.

With the doses administered in the present study the increase in K_{a} during spironolactone therapy was considerably higher than the increase obtained by potassium supplement ($p < 0.01$). Although the rise in K_{a} might seem higher with potassium supplement, the difference however is not significant ($p > 0.1$). The changes in K_{acc} did not differ in the two groups.

There was a significant though not particularly pronounced correlation between K_{a} /m² and K_{e} after thiazide therapy ($r = 0.3490$, $p < 0.05$, $n = 34$).

The interrelation between the changes in K_{acc} and K_{e} was not particularly pronounced but will be described in another paper (13).

Very few side-effects were observed during the examination period. Only one patient developed symptoms of hypokalaemia such as pronounced

fatigue and muscle weakness. These symptoms occurred when only hydrochlorothiazide was given, and K_{a} was then 1.9 mEq/l. Three females suffered from postmenopausal menstruation-like bleeding during spironolactone therapy and as previously mentioned one patient was excluded for that reason.

DISCUSSION

Following thiazide therapy without potassium supplement or administration of potassium-sparing diuretics for at least 4 weeks the K_{e} was significantly lower than the levels found in patients with untreated essential hypertension, determined by the same method and in the same laboratory (6). As stated in the introduction this is with a few exceptions consistent with findings made in numerous other studies. The females included in the present study seemed to be more potassium depleted than the males. It appears from Table I that total potassium in males and females treated with thiazide was 90% and 80% respectively of K_{e} in untreated subjects. Correspondingly K_{a} as shown by median value and interquartile range was 3.3 mEq/l (3.1-3.5) and 3.0 mEq/l (2.7-3.2) respectively ($p < 0.01$). The average weight of males and females being 83.0 and 63.1 kg respectively the most probable explanation is that the females received "relative over-dosage" as compared with the males. Also the K_{acc} levels were lower than those found in normal subjects and this supports the theory that there must be an intracellular deficiency. This is in accordance with the findings of Boyd (4).

With a potassium supplement

the total potassium increased by about 11%. This change was almost of the same order of magnitude as the deficiency during thiazide therapy found by other authors (2, 11, 17). The increase in K_{ex} showed also that potassium was retained in the cells. The change in K_{ex} was slight but significant, and in accordance with that found in other studies (6, 11) although the increases found in several investigations were not significant (1, 14, 15).

The change in total potassium observed was consistent with the findings made in several studies (11, 15) but differed from others (1, 14). Investigations of patients with chronic heart diseases who were treated with diuretics have also presented contradictory results (10, 20). These discrepancies do not seem to be due to the different methods employed. In studies where K_{ex} was determined by means of ^{42}K (6, 10, 15) and in investigations determining total potassium by whole-body counting of ^{40}K (1, 11, 14) both increased total potassium and unchanged levels have been found. In the studies where potassium depletion occurred after thiazide treatment, and where potassium supplement was given a significant rise in total potassium was found; this finding accords with the present study (11, 15, 20). On

the other hand investigators who did not observe a change in the total potassium during treatment with diuretics found no changes following potassium supplement either (1, 6, 10, 14). Possibly the contradictory findings are partly attributable to dosage of diuretics and potassium chloride dietary differences may also exert an influence (2). The potassium supplement dosage given during the present study was higher than that used in other investigations.

Spironolactone supplement increased K_{ex} significantly about 6%. This was consistent with the findings in other studies (6, 15) and it has also been proved that spironolactone produces an increase in K_{ex} in patients with primary hyperaldosteronism (5). In a study in which patients with essential hypertension were given only spironolactone, a significant reduction in K_{ex} was found but also an increase in K_{ex} (21) apparently these patients were not potassium-depleted. The increase in K_{ex} found during the present study supports the above mentioned results.

The increase in K_{ex} found in the spironolactone group was considerable and higher than that found

in the potassium chloride group. On the other hand the change in K_{ex} was not markedly different in the two groups. Spironolactone is a specific aldosterone antagonist and inhibits the renal potassium excretion resulting from aldosterone. The results could also indicate that spironolactone exerts an influence on extrarenal cell membranes resulting in a changed distribution of potassium between the extracellular and intracellular compartments, although there are no definite indications that aldosterone and aldosterone antagonists influence other than epithelial surfaces (7, 8, 16). However this does not exclude the possibility that spironolactone may possess important pharmacological properties independent of aldosterone. Studies on isolated carcinoma cells and human erythrocytes have shown that spironolactone in the absence of aldosterone increases intracellular sodium and decreases potassium (7). Similarly the sodium transport across isolated frog skin is inhibited by spironolactone in the presence as well as absence of aldosterone (8, 16).

Whereas BP was not influenced by potassium supplement, a moderate but significant fall was observed both in the systolic and the diastolic BP during spironolactone therapy even though the BP was well controlled when hydrochlorothiazide alone was given. Spironolactone exerts its most pronounced hypotensive effect in patients with low or hyporesponsive plasma renin activity, i.e. patients with primary hyperaldosteronism or other mineralocorticoid excess and 20–30% of patients with essential hypertension (9, 18). Practically all such patients will be normotensive when treated solely with high doses of spironolactone 400 mg daily (5, 9, 18). However as proved by the present study and by other investigations (9, 18, 22) lower doses of spironolactone exert a moderate more non-specific hypotensive effect, which can be compared to that of thiazides, and which is additive when combined with other diuretics (22).

It has often been maintained that serum potassium is a poor expression of the potassium balance. However a significant although not pronounced positive correlation between K_{ex}/m^2 and K_{ex} following thiazide therapy was found. Edmonds and Jasmi (11) also found such a correlation.

It is concluded that, in patients with potassium depletion resulting from treatment with diuretics

it is possible to increase both the extracellular and the intracellular potassium by adding potassium chloride or spironolactone. On the other hand it is difficult to draw any definite conclusions as to the necessity of such supplement. It is agreed that, under certain circumstances, it is important to avoid potassium depletion, e.g. in patients with liver cirrhosis or diabetes mellitus and in patients on digitalis. Whether it is necessary as a routine to give a potassium supplement or a potassium-sparing diuretic to hypertensive patients on thiazide therapy cannot however be considered to be clarified. In such patients symptoms which can with certainty be associated with hypokalaemia are extremely rare (11-12). It is well known however that severe potassium deficiency may result in damage to renal tissue and the myocardium, disturbances in the tubular function of the kidneys and in a great many enzymatic reactions, although studies in animals and man could indicate that such damage is not observed until the deficiency is about 20-40% of the total potassium (19). The reduction in total potassium which is commonly seen during long-term diuretic therapy in patients with arterial hypertension without cardiac complications ranges between 8 and 14% (2, 11-12). Hence Healy et al. (17) were unable to find changes in the tubular function of the kidneys or in the carbohydrate metabolism. In the present study the females receiving thiazide treatment had an average k_{ex} of 3.0 mEq/l (interquartile range 2.7-3.4) and a reduction in total potassium of about 20%. Consequently such potassium deficiencies should be corrected. Whether one drug should be preferred to another during treatment depends on the effect desired. Potassium chloride is at least as effective as spironolactone for increasing total potassium, but if a severe hypokalaemia is to be corrected spironolactone is to be preferred. Moreover it has an additive diuretic and hypotensive effect.

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HEMODYNAMIC EFFECTS OF ACUTE AND PROLONGED β -ADRENERGIC BLOCKADE IN ESSENTIAL HYPERTENSION

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Abstract Hemodynamic studies have been performed in 15 male patients with mild to moderately severe essential hypertension. Studies were made after four weeks of placebo treatment and again after acute intravenous administration of propranolol (0.22 mg/kg) and after four weeks of oral propranolol therapy (160-320 mg daily). After acute β -adrenergic blockade a significant reduction of cardiac index (CI) occurred, while total peripheral resistance (TPR) increased significantly resulting in unaltered BP. After four weeks of oral propranolol the CI remained significantly reduced, but TPR was readjusted towards the initial level, resulting in reduction of BP in most patients. Major reductions of stimulated plasma renin activity were seen, but these were not correlated to the changes of BP, CI or TPR. Studies of the baroreceptor reflex by means of administration of angiotensin suggested a change towards increased sensitivity although this change did not achieve statistical significance.

Antihypertensive effect of propranolol has been reported in several studies (6, 8, 11, 15, 20, 23). As most forms of established hypertension are characterized by increased total peripheral vascular resistance (TPR) and normal cardiac output (CO) (5, 11, 17), the antihypertensive properties of propranolol have been hard to explain in view of its predominantly cardiac effects. Several suggestions have been offered as tentative explanations e.g. resetting of baroreceptors (15), central nervous effects (6), adaptation of vascular resistance in response to chronic reduction of CO (20) and local anesthetic effects on vasoconstrictor fibers (4). Lately propranolol-induced reduction of plasma renin activity (PRA) in hypertensive patients

has been demonstrated (9) which may be of importance in the hypotensive mechanism especially in conditions with high plasma renin. As the effects of acute and chronic administration of propranolol are different particularly in regard to the effect on BP (16, 20, 21) further confusion has resulted.

For this reason the present study was designed with the intention of studying the antihypertensive effect of propranolol, particularly the hemodynamic alterations resulting from acute and chronic β -adrenergic blockade. We were interested to see whether TPR would readjust in the presence of continuously reduced CO. In addition, special interest was directed to the study of baroreceptor sensitivity and whether a change would take place. Finally PRA was studied with the intention of exploring connections between changes of renin and changes of peripheral vascular resistance.

MATERIAL AND METHODS

Fifteen male patients with mild to moderate essential hypertension were studied. Their average age was 44 years (range 26-60). The average known duration of hypertension was 6.5 years (range 0-17). Before entering the study all patients but two were receiving antihypertensive therapy usually consisting of a-methyldopa and/or diuretics.

Secondary causes of hypertension were excluded in all patients by thorough clinical examination, rapid sequence pyelogram and laboratory studies (serum electrolytes, PRA and urinary excretion of aldosterone, epinephrine and norepinephrine). In addition ECG, chest X-ray, urine microscopy, serum creatinine, serum lipids and liver function were checked repeatedly during the study.

All patients were told of the investigational nature of the study and gave their informed consent to participation in it.

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Plan of study

After stopping all previous therapy the patients were given placebo treatment in a single blind fashion on an outpatient basis. Placebo treatment continued for four weeks after which the patients were hospitalized for baseline studies including the first hemodynamic study. They were discharged after four days and were put on oral propranolol 40 mg four times daily as the sole antihypertensive treatment. After two weeks this dosage was increased to 80 mg four times daily unless a diastolic BP reduction of 15 mmHg or more was observed. Treatment with propranolol was continued for another two-week period. The average daily propranolol dosage during weeks 3 and 4 was 230 mg. After four weeks of oral propranolol treatment a repeated hemodynamic study was performed.

Hemodynamic methods

The hemodynamic studies were performed in the morning with the patients fasting.

Systemic arterial pressure was measured in the brachial artery by a Statham strain gauge receptor (P 23 G). Mean arterial pressure (MAP) was calculated as the diastolic BP + 1/3 of the pulse pressure.

Q was determined by the indicator dilution technique (12) using a Gilford densitometer and indocyanine green (Cardio-Green).

Respiratory movements were monitored continuously by means of 2 bellows attached around the chest and connected to a strain gauge by a rubber tube. The standard leads of the ECG were connected and lead I was recorded continuously on a Gilson polygraph together with intraarterial BP respiration and dye curves.

Stroke volume (SV) was calculated by dividing Q by heart rate (HR). TPR was obtained by dividing MAP by Q and was expressed in arbitrary units (U). Q, TPR and SV were corrected for body surface area and the corresponding indices QI, TPRi and SVi were derived.

PRA was measured with a radioimmunoassay technique (7) in the initial untreated state during tilt (stimulated) and again in the tilted position after acute and prolonged β -adrenergic blockade.

Hemodynamic study I

The first resting Q was determined 15 min after all catheters were placed. The patients were then tilted to a 45° head-up position for 10 min and Q and PRA were determined. Approximately 10 min after resumption and under resting conditions the baroreceptor reflex was assessed. This was done by rapid i.v. administration of angiotensin (Hypertensin Ciba) usually 1.5 μ g, while recording changes in BP and HR. Systolic BP was plotted against the corresponding R-R interval in the ECG using the technique of Bristow et al. (1). Plotting was continued until the pressure peak occurred. No data during inspiration were used. Usually 15–30 points could be plotted in this way for each patient. The points were then analysed with a standard correlation test and only if a statistically significant correlation resulted ($p < 0.05$) was the BP/R-R slope used for further comparisons.

Propranolol was then administered intravenously 0.05 mg/kg b.wt. over 2 min. Q was determined 8 min after the completion of the injection. A additional amount of propranolol 0.05 mg/kg. was then given and Q measurements were repeated after an identical interval. Finally a third injection of 0.12 mg propranolol/kg was given, bringing the total dosage to 0.22 mg/kg. Hemodynamic determinations were then repeated. The 10-min 45° head-up tilt was then repeated, at the end of which hemodynamics were determined.

Hemodynamic study II

The second hemodynamic study was done at the same time of day and under identical conditions as the first study but following four weeks of oral propranolol treatment. The same studies were repeated with the exception that i.v. propranolol was not given and only one 10-min period of tilt with Q and PRA measurements was performed.

Statistically significant BP/R-R slopes were obtained at both the first and second hemodynamic study in 7 patients. Only data from these 7 patients were used for comparisons (paired *t*-test).

Assessment of β -adrenergic blockade

The degree of β -adrenergic blockade was tested three times during the study by continuous i.v. infusion of a β -adrenergic stimulant—Isoproterenol—while monitoring HR on the ECG. On each occasion HR was determined before the start of the infusion. Isoproterenol was then given in constant i.v. infusion of 3 μ g/ml at the rate of 1 ml/min for 3 min. The response to β -adrenergic stimulation was taken as the difference between the final HR and the HR before the start of the infusion.

The first assessment of response to isoproterenol was made after four weeks of oral placebo treatment before any propranolol had been given. The second determination was made after i.v. administration of 0.22 mg propranolol/kg, and the final assessment after four weeks of oral propranolol therapy and approximately 8 hours after the last dose.

Plasma propranolol determinations

Plasma propranolol concentration was determined by fluorimetric technique (18) by Ayerst Laboratories, Montreal, Canada. Blood samples were drawn 70 min after the completion of the i.v. injection of propranolol and again following four weeks of oral treatment, approximately 90 min after the last dose. The plasma was separated by centrifugation and chilled to 4°C before being sent in an isolated tube for analysis.

Statistical methods

Student's *t*-test, the *t*-test for paired observations and correlation coefficients were calculated using the Mids (Constant) computer program of the Statistical Research Laboratory at the University of Michigan.

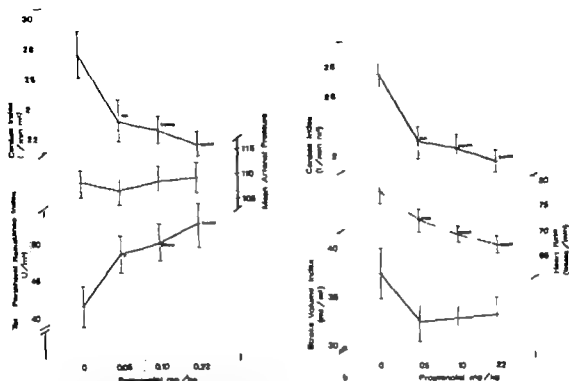


Fig 1 and b Effect of increasing doses of L. propranolol (mean \pm S.E.M.). The cumulated doses on the x-axis were given with 10-min intervals. Statistical

comparison (paired *t*-test) is with initial values.
 $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$ **** $p < 0.001$

RESULTS

Effect of intravenous propranolol

As illustrated in Fig. 1a even 0.05 mg/kg of i.v. propranolol caused a marked drop of Q_i from ~ 76 to 2.29 l/min m^2 ($p < 0.01$). TPRI increased from 41.3 to 48.5 U/ m^2 ($p < 0.05$) thereby minimizing the effect on MAP which remained virtually unchanged (108.3 vs. 107.2 mmHg, $p > 0.25$).

Further administration of propranolol to a total dosage of 0.10 and 0.22 mg/kg, respectively caused further non-significant reductions of Q_i

and increments of TPRI while MAP remained unchanged (Fig. 1a). The overall hemodynamic effects are given in Table I. As illustrated in Fig. 1b the reduction of Q_i was mainly the effect of decreased HR, while SVI did not change significantly.

Effect of four weeks of oral propranolol therapy

The effect of four weeks of oral propranolol therapy was similar to the acute effects of i.v. propranolol in that Q_i remained at a significantly lower level than initially albeit the reduction was

Table I. Hemodynamic changes at rest

	Propranolol				Orally ^b	Difference ^c		<i>p</i> <
	Placebo	L	Difference ^a	<i>p</i> <				
Q _i (l/min-m ²)	2.76	2.18	0.58	0.001	2.35	0.41	0.01	
MAP (mmHg)	108.3	109.9	1.7	n.s.	102.1	6.2	0.05	
TPRI (U/m ²)	41.3	52.3	11.0	0.005	43.9	1.6	n.s.	
HR (beats/min)	76.0	67.1	8.9	0.0001	79.6	16.4	0.0001	
SVI (ml/m ²)	37.3	33.0	4.3	n.s.	40.1	2.9	n.s.	

0.22 mg/kg. For 4 weeks, 160–320 mg/day Compared to measurement after placebo.

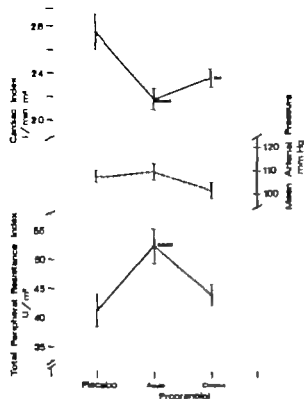
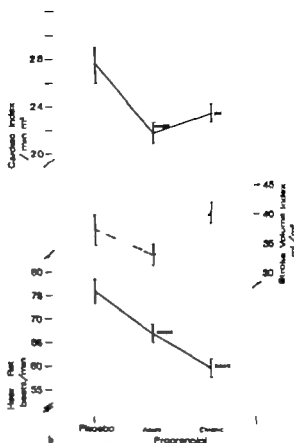


Fig 2a and b Effects of acute i.v. and chronic oral administration of propranolol (mean \pm S.E.M.) Statistics as in Fig. 1



of as pronounced (Table 1 Fig. 2a). The reduction of Q_i was mainly an effect of reduced HR, whereas SV_i did not change significantly (Fig. 2b).

However in contrast to the effect of acute β -adrenergic blockade there now was a reduction of MAP (Fig. 2a). TPRI was correspondingly less elevated than after acute propranolol and as a matter of fact was not significantly higher than in the untreated state.

Analysis of the hemodynamic changes in individual patients revealed that, while 12 patients showed reduced MAP after four weeks of propranolol therapy 3 showed no decrease or even an increased MAP. For this reason the 12 responders were compared to the 3 non-responders.

Already after acute i.v. propranolol there was a significant difference in MAP between the two groups (MAP 105.9 vs. 121.4 mmHg, $p < 0.05$) (Fig. 3). As expected this difference was even more pronounced after four weeks of oral propranolol (MAP 97.1 vs. 122.2 mmHg, $p < 0.001$). There were no significant differences between responders and non-responders regarding Q_i but

after four weeks of oral propranolol responders had significantly lower TPRI (42.3 vs. 50.2 U, $p < 0.05$) (Fig. 3). Differences in HR and SV_i were insignificant both after acute and chronic β -adrenergic blockade.

Effects of tilt

The hemodynamic effects of tilt in the untreated state after acute i.v. propranolol and after 4 weeks of oral therapy are presented in Table II. Propranolol blocked the HR response to tilt both after acute and chronic administration. The well known reduction of Q during tilt was observed also after acute and to a lesser extent after chronic β -adrenergic blockade. Finally the chronic β -adrenergic blockade did not interfere with the homeostasis of BP during tilt. Significant increases of MAP were seen in this situation both after placebo and after 4 weeks of oral propranolol. On the other hand a non-significant reduction of MAP occurred after acute i.v. propranolol.

Effect on plasma renin activity

PRA after tilt in the untreated state after acute

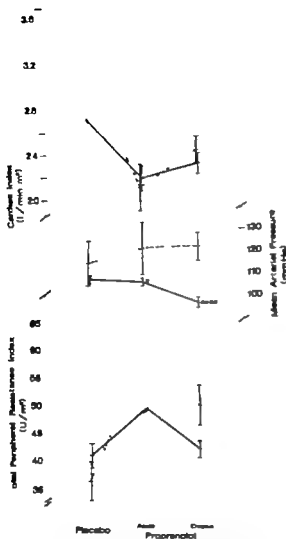


Fig. 3 Effect of acute and chronic oral administration of propranolol (mean \pm S.E.M.). Statistical comparison is between responders (—) and non-responders (---) (Student's *t*-test). Probabilities as in Fig. 1

Table 11. Hemodynamic changes during tilt

	Placebo (<i>n</i> =15)				I.v. propranolol* (<i>n</i> =12)				Oral propranolol* (<i>n</i> =12)			
	Rest	Tilt	Difference	<i>p</i> <	Rest	Tilt	Difference	<i>p</i> <	Rest	Tilt	Difference	<i>p</i> <
Q _t (l/min/m ²)	2.76	2.19	0.57	0.005	2.19	1.60	0.59	0.0005	2.44	2.04	0.40	0.005
MAP (mmHg)	106.3	119.6	13.3	0.0005	106.4	98.3	8.1	n.s.	103.4	110.3	6.9	0.005
TPRI (U/m ²)	41.3	46.5	5.2	0.0001	50.4	63.8	13.4	0.005	42.6	54.6	12.0	0.0005
HR (beats/min)	76.0	84.9	8.9	0.005	69.5	69.8	0.3	n.s.	57.7	60.6	2.9	0.01
SVI (ml/m ²)	37.3	26.4	10.9	0.0001	32.0	22.9	9.1	0.0001	42.7	34.4	8.3	0.0005

0.22 mg/kg For 4 weeks, 160–320 mg/d (average 270 mg)

i.v. propranolol and after 4 weeks of oral therapy are presented in Table III. The effect on PRA of chronic propranolol therapy was striking, causing a reduction of stimulated (tilted) PRA by 85% (from 749 to 39 ng/100 ml/h $p<0.005$). However the reduction of PRA was not significantly correlated to the change of MAP, TPRI or Q_t either in the recumbent or tilted position (correlation coefficients 0.077–0.104 $p<0.25$). Neither was the change of PRA, expressed in percent, significantly correlated to the percentage change of MAP, TPRI or Q_t either recumbent or tilted (correlation coefficients 0.000–0.387 $p>0.10$). There was no difference between responders and non-responders in the reduction of PRA, the three non-responders showing reductions of 81, 90 and 96% respectively as compared to 85% for the entire group.

Effect on baroreceptor reflex

Only in 7 patients was a statistically significant correlation between systolic BP and R–R interval (BP/R–R slope) obtained after angiotensin injection both in the untreated state and after four weeks of oral propranolol. The average BP/R–R slope in the untreated state was 4.84 and after oral propranolol 7.64. The difference 2.80 ± 1.06 did not quite achieve statistical significance ($p=0.115$) (paired *t*-test).

Effect of isoproterenol

As described before, infusion of isoproterenol was used to assess the degree of β -adrenergic blockade in all patients. In the initial untreated state the HR increased by 39.4 beats/min ($p<0.0001$) in response to the i.v. infusion of $3 \mu\text{g/min}$. There was no difference between responders (39.8) and non-responders (38.0) in this respect. After acute

Table III PRA before and after β -adrenergic blockade

PRA (ng/100 ml/h) tilt

Placebo	I.v. propranolol (0.22 mg/kg)	Oral propranolol (160-320 mg/d)
248.7 \pm 69.8	204.7 \pm 70.1	39.0 \pm 10.1
$p < 0.01$		$p < 0.005$

i.v. administration of propranolol and repeated isoproterenol infusion at the rate of 3 μ g/min the HR did not increase significantly (16 beats/min). Finally following four weeks of oral propranolol the response to isoproterenol infusion was 5.3 beats/min (the increase in responders being 6.1 and in non-responders 2.0 beats/min) indicating that all patients had a significant degree of β -adrenergic blockade.

Plasma propranolol determinations

Average plasma propranolol concentration after acute i.v. administration of 0.22 mg propranolol/kg was 100.5 \pm 6.0 ng/ml and individual variations were small. Following four weeks of oral propranolol treatment, at either 160 or 320 mg daily the average plasma propranolol concentration was 123.1 \pm 46.1 ng/ml. More than ten-fold variations between individual patients were observed. Correlations between the various hemodynamic and PRA changes and plasma propranolol concentrations were not statistically significant either after acute i.v. or after prolonged oral administration of propranolol. Nor was the response to isoproterenol infusion significantly correlated to plasma propranolol concentration.

DISCUSSION

The effect of propranolol was studied in 15 men with mild to moderately severe essential hypertension. In agreement with previously published results (6, 8, 14, 15, 20, 23) a BP reduction was seen in most cases following oral treatment.

Hemodynamically it was obvious that the i.v. administration of even 0.05 mg propranolol/kg caused a significant reduction of HR and Q while the decrease of SVI did not achieve statistical significance (Figs. 1 and 2). Doubling and quadrupling the dose further decreased HR and Q but the importance of these increments of dosage was minor.

Just as important was the finding that BP was not affected by this acute decrease of Q. Consequently the calculated TPR had increased to a degree corresponding to the decrement of Q. Obviously this in the effect which from a theoretical point of view should be expected from a β -adrenergic blocking agent.

After four weeks of oral administration of propranolol the Q and HR were still decreased. In contrast to the acute situation however the majority of patients now showed reduced BP with an accompanying reduction of TPR. This finding is in agreement with recent observations of others (20).

It is tempting to speculate about the underlying mechanisms explaining this phenomenon particularly the question of why a chronic reduction of Q would cause a readjustment of TPR in most patients. Resetting of baroreceptors has been suggested (15) and our data suggest a change of baroreceptors to a more sensitive setting. For a given increase of systolic BP a comparatively greater reduction of HR occurred after treatment. However it should be stressed that we could not demonstrate a significant change of baroreceptor sensitivity possibly due to the small number of patients available for this comparison. Further more it should be pointed out that a comparison to baroreceptor sensitivity was not made after acute β -adrenergic blockade. However studies of the baroreceptor sensitivity using the same technique as in the present study although revealing some gain of reflex sensitivity after i.v. propranolol, showed that atropinization completely abolished the HR response to BP elevation (19). This would indicate that vagal effects are mainly responsible for the baroreceptor-mediated reduction of HR and consequently it would be justified to compare the sensitivity in the untreated state in that after prolonged oral β -adrenergic blockade. Preliminary unpublished data of our own reveal that acute i.v. administration of propranolol 0.22 mg/kg does not significantly alter the BP/R-R slope.

Our tilt results do not support the opinion by Oparil et al (13) that renin plays an important role in the hemodynamic adjustments associated with postural changes. The vascular response to tilt after prolonged β -adrenergic blockade was not impaired in spite of marked reduction of PRA. It would therefore seem that renin/angiotensin is not

directly responsible for the hemodynamic alterations during the acute change of posture.

The hypothesis that the hypotensive effect of propranolol is secondary to its local anesthetic effect and that in fact the reduction of BP would follow from anesthesia of vasoconstrictor fibers (4) is most interesting. However, it is hard to explain the different response to acute and chronic therapy using this explanation. Furthermore studies in man, using the dextranomer of propranolol which has no β -adrenergic blocking effect but retains the local anesthetic effect, have shown no hypotensive effect (2.). Tarazi and Dustan (20) were the first to publish results indicating that reduction of BP during propranolol treatment follows from a long-term adaptation of peripheral resistance. Obviously our results are in full agreement with their statement, even if at present we are not able to explain the mechanism underlying this adjustment.

The marked reductions of stimulated PRA, and therefore logically also of angiotensin II, could theoretically reduce peripheral vascular resistance. However the renin/angiotensin mechanism is not considered to be of etiological importance in mild essential hypertension, and PRA is usually normal or even low in this condition (3, 10). Our results are in agreement with such a view as no significant correlation was seen between the change of PRA and the changes of MAP, Q and most importantly TPR. Furthermore the reduction of PRA was equally pronounced in the three patients who showed no decrease of MAP. This indicates that reduction of PRA is not the major determinant of the antihypertensive mechanism of propranolol in mild essential hypertension. The lack of correlation between the change of PRA and the change of TPR indicates that the readjustment of peripheral vascular resistance that takes place during prolonged treatment with propranolol is independent of the change of PRA.

Plasma propranolol concentration was not found to be correlated to either the hemodynamic changes or the effects of i.v. infusion of isoproterenol which is in agreement with recent observations by others (24). This could indicate that tissue binding of propranolol is of greater importance than the plasma concentration. Other possibilities are that active metabolites e.g. 4-hydroxypropranolol, contribute significantly to the degree of β -adrenergic blockade.

Finally the response to isoproterenol infusion failed to single out non-responders. However we do not wish to draw any conclusions from this as individual dose-response curves before and after propranolol were not made.

CONCLUSIONS

1 Acute β -adrenergic blockade does not cause reduction of BP in patients with essential hypertension. The reduction of Q in this situation is compensated by a corresponding increase of TPR, thereby maintaining BP unaltered.

2 After four weeks of β -adrenergic blockade the Q is still reduced but, due to an adjustment of TPR, the BP is significantly reduced in most patients.

3 The alterations of TPR and BP are not directly dependent on the marked reductions of stimulated PRA.

4 An increase of the BP/R-R slope although not statistically significant, indicated that baroreceptors may have been reset towards a greater sensitivity thereby suggesting the mechanism whereby peripheral resistance is adjusted.

ACKNOWLEDGEMENTS

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CEREBROVASCULAR LESIONS

Active Antihypertensive Treatment and the Present Situation

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Abstract Active antihypertensive treatment has now been carried out in Sweden for more than 23 years. It has, however, been reasonably widespread only for about 15 years. Increasingly efficient heart valve operations have been available for nearly 20 years, with a decline in the number of advanced mitral valvular defects. A downward trend for cerebral vascular lesions from 1950 to 1964 in Göteborg has earlier been noted. A new investigation for the year 1968 shows the same tendency for cerebral haemorrhages and cerebral embolism, but unchanged figures for encephalomalaciae. In 1968 as in 1964 considerable number of patients with known hypertension were on inadequate therapy or had been permitted to drop treatment completely.

Active antihypertensive treatment in its present meaning has now been available for over 20 years. After 1950 a dramatic change has occurred with a successive decrease of incidence and mortality from cerebrovascular lesions due to hypertension. Earlier publications by Hood et al. (1, 2, 3, 4, 5) have analysed the successive changes of cerebrovascular lesions and active antihypertensive treatment from 1950 onwards to 1964. A considerable reduction both in incidence and mortality has been noted in a population supposed to be actively treated for hypertension.

It was shown that most complications appeared in patients with poor treatment or none at all. Even in 1964 there was a considerable number of patients with hypertension not subjected to active treatment. The question is, then, whether the panorama has changed with time and with improved possibilities for survey, diagnosis and treatment and whether there may be still more to gain. In order to answer this question the present study was carried out.

MATERIAL AND METHODS

The records from the Departments of Medicine and Neurology at Sahlgren's Hospital in Göteborg, Sweden, were sought for patients admitted in 1968 with acute cerebrovascular lesion. These are the same departments as those in which the analysis quoted above was carried out. Sahlgren's Hospital is the only hospital with acute admission wards within the area. As in the quoted material, patients older than 70 years, those belonging to other county council areas and those with recurrent stroke were excluded. To give clearest diagnostic delineation the analyses were also confined to those dying of their cerebrovascular lesion or to cases in which the lesion led to a permanent sequela.

An attempt was made to divide the material, consisting of 111 patients, into the groups cerebral haemorrhage ($n=20$), encephalomalacia ($n=79$) and cerebral embolism ($n=12$).

The diagnosis of cerebral haemorrhage was based upon autopsy, cerebral angiography and/or clinical history strongly suggestive of haemorrhage. As complement hammer percussion was performed. Cerebral embolism was diagnosed upon suggestive clinical history and a reasonable source of embolism. In the absence of a typical onset or of heart disorder causing embolization the majority were considered to be encephalomalaciae.

Diastolic BP on admission was registered. Further information about eyeglasses, ECG laboratory findings and other information of interest were noted. Of special interest was information concerning previous known hypertension, its treatment, symptoms suggesting hypertension and coexisting disease of importance or complicating conditions. The definition of actively treated hypertension as earlier used by our group requires treatment with at least two drugs or when single drug is used, a diastolic BP below 100 mmHg (6).

As the age distribution is constantly changing, the number of patients has been recalculated corresponding to the number per 10000 inhabitants at risk in each age group.

RESULTS

The age distribution is shown in Fig. 1 where also the corresponding figures for the years 1949 and 1964 are shown (5). The absolute numbers for the population at risk for each age group in 1968 are also given (7). For all diagnostic groups there is a considerably larger number within the highest age group. The corresponding figures for the whole material are given in Fig. 2. It shows that compared with 1964, there is a further decrease for cerebral haemorrhage and embolism while for encephalomalacia the number is unchanged.

An analysis of symptoms and signs in patients with haemorrhage and encephalomalacia is shown in Table I. About three-quarters of the patients with haemorrhage but not half of those with encephalomalacia had known hypertension prior to the attack. However, symptoms suggesting hypertension were present in a considerable additional part of the group with encephalomalacia while this was not the case amongst those with haemorrhage. A history of positive heredity for cardiovascular disease was obtained in 50% of the encephalomalacias and 37% of the haemorrhages.

In the whole material only one patient with backgrounds corresponding to grade IV and three to grade III according to Keith et al. were found.

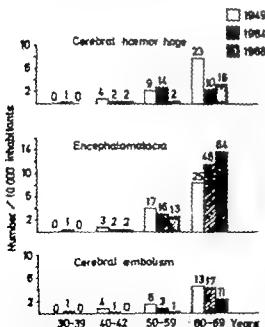


Fig. 1 Age distribution of patients with first attack of cerebrovascular lesion leading to permanent disability or death.

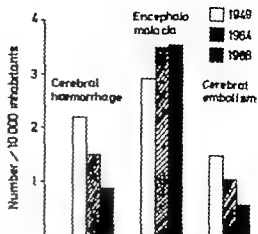


Fig. 2 Diagnostic breakdowns of first attack of cerebrovascular lesion leading to permanent disability or death.

A survey of other complicating factors on admission in the patients with stroke is given in Table II. In the group with cerebral haemorrhage there were no conditions involving a haemorrhagic tendency but three patients presented with other serious complications, viz. one with other vascular disease, one was diabetic and one chronic alcoholic. The only three surviving patients with haemorrhage were the ethylist, who had a normal BP and two patients without complicating factors and a diastolic BP on admission of 120 and 110 mmHg respectively whose antihypertensive treatment had been dropped or inadequate. A substantially greater number of complicating factors both of haemorrhagic type (dicoumarol treatment) and non-haemorrhagic type (diabetes, known hyperlipidaemia, chronic alcoholism, angina pectoris, claudicatio intermittens, uraemia and syphilis) were found amongst the encephalomalacias.

When cholesterol and triglyceride were determined during fasting directly after admission an elevated value was supposed to be significant for hyperlipidaemia. For the patients with encephalomalacia, sampling according to those conditions was done for cholesterol in 50% and for triglyceride in 39%. Among the latter the levels were elevated (cholesterol ≥ 300 mg/100 ml triglyceride ≥ 2.0 mmol/l) in 37 and 23% respectively. Manifest diabetes was present in 14%, the same figure as obtained in the earlier material by Hood et al. (5).

An analysis of the cases of cerebral embolism showed that valvular heart disease with or without arrhythmia was the possible cause in five pa-

Table 1 Analysis of symptoms and antihypertensive treatment prior to cerebrovascular lesion

	Haemorrhage		Encephalomalacia	
	(n)	(%)	(n)	(%)
Anticoagents not obtained	4	20	7	9
History blank	3	15	29	37
Acute episode (earlier)			4	6
Continuous symptoms (headache, trigeminal pectoris, breathlessness)			5	6
Known hypertension	13	65	33	42
Total	20		79	
Active hypertensive treatment				
Never tried	4		6	
Tried but dropped	3		10	
Maintained but inadequate	3		10	
Maintained	3		7	

lients the other seven all had arrhythmia due to arteriosclerotic heart disease. There was only one patient with operated valvular disease (mitral stenosis and insufficiency). Prophylactic dicumarol treatment had been given to three patients but in one of them had therapeutic anticoagulatory levels been achieved.

DISCUSSION

The results show a further decrease of cerebral haemorrhage when recalculated upon the whole population at risk. The number of cerebral embolism was about the same as in 1964.

When considering the age distribution, it is seen that a reduction has taken place in the ages below 60 for both haemorrhages and encephalomalacias. Above the age of 60 there was an increase indicating that the incidence of stroke has been pushed upwards in age. There were scarcely any cerebrovascular lesions at all below 50 years. Within the age group 60-69 there is still a tendency to progression towards the higher age as most of the cases were aged 65-69.

One source of error in this investigation may be that the records of the Coroner's Office have not been sought for deaths due to cerebrovascular lesions. However most people live some time after a stroke and are then admitted to hospital

especially in a city with a well developed ambulance service as in Göteborg. It must also be noted that figures from the Coroner's Office were not included in the previous clinical investigation here used for comparison. The minor number of patients who die in their homes and have a death certificate without autopsy from their physician may be supposed to be unchanged from 1949 to 1964.

A downward trend for the mortality in stroke was noted even before the era of active antihypertensive treatment due to improved cure antibiotics, etc. This however does not interfere with the results of this investigation.

It is a striking fact that still many people with known hypertension either have never been treated or the treatment has been allowed to get completely out of order (Fig. 3). The reasons may be lack of initial care and determination in treatment or aggravating factors such as alcoholism or psychiatric abnormality of the patient. One patient forgot his medicine when going on a journey, two others stopped the treatment because the medicine stores were not refilled. Even when treatment was continued, it must still be considered really inadequate in many patients.

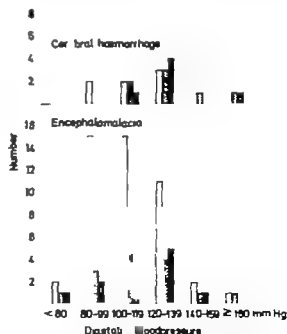


Fig. 3 Diastolic BP on admission and antihypertensive treatment in the 1968 series. □=previous BP unknown or nonmeasurable. ▨=treatment never tried or dropped. ■=treatment maintained.

Table II Analysis of complicating conditions previous antihypertensive treatment and mean diastolic BP on admission in patients with cerebral haemorrhage and encephalomalacia

Number of deaths given within parentheses

	Antihypertensive treatment maintained		Antihypertensive treatment dropped or never tried		Previous BP unknown or normal	
	No. of pts.	Diastolic BP	No. of pts.	Diastolic BP	No. of pts.	Diastolic BP
Haemorrhage						
No complicating conditions	4 (3)	105	6 (5)	120	3 (5)	105
Non-haemorrhagic complications	2 (2)	115	1 (1)	120	2 (1)	110
Encephalomalacia						
No complicating conditions	13	112	3 (2)	110	43 (3)	90
Haemorrhagic complications	2	107	1	100		
Non-haemorrhagic complications	2	110	12 (1)	105	3	100

Valvular heart disease contributed to nearly half of the cases with embolism. Only one operated patient was found in this group. Earlier investigations by Wood (9) have shown that 9-14% of patients suffering from rheumatic valvular disease have a history of embolism, 60% of them are cerebral.

It is striking that all cases of embolism, regardless of origin occurred in patients without or with inadequate anticoagulant treatment. In an analysis of 734 patients with rheumatic heart disease Szekely (8) found that anticoagulant treatment reduced the incidence of embolic recurrences. The results of the present investigation also stress the importance of adequate embolic prophylaxis.

Uræmia was found only in two cases neither of them was fatal. There may be many possible explanations of this. A more active treatment of hypertension may be the principal reason. Earlier tracing and treatment of pyelonephritis and glomerulonephritis are also certainly of importance. The restricted purchase of phenacetin-containing analgesics has undoubtedly reduced the frequency of non-obstructive pyelonephritis.

The still continuing trend of decreased incidence of stroke after more than two decades of available active antihypertensive treatment is promising. But it is a sad reality that so many patients are still not satisfactorily treated. It is reasonable to conclude that a far greater benefit is to be gained from an active aggressive attitude in the treatment

of hypertension as well as other predisposing factors.

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INSULIN RESPONSE TO INTRAVENOUS GLUCOSE DURING LONG-TERM TREATMENT WITH PROPRANOLOL

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Abstract The early insulin response to *L.* glucose has been studied in 10 patients with primary hypertension before and during long-term treatment with large oral dosage of propranolol. No change in the early insulin secretion was found.

In recent years the β -adrenergic blocking agents have become widely used in the treatment of hypertension. These drugs are well tolerated by the patients. No serious adverse metabolic effects have so far been noted but propranolol has been reported to attenuate the acute insulin response to glucose (1-3, 4). This might be of interest since a defective early insulin response has been postulated to be characteristic of prediabetes (2).

The aim of the present study was to investigate whether any change in insulin response to glucose occurs during long-term treatment with large doses of propranolol.

MATERIAL

The material consisted of 10 men, aged 50, with hypertension discovered at health examination survey. The criteria for hypertension was a diastolic BP of ≥ 105 mmHg on at least two occasions in recumbent position. BPs were measured in the morning after 10 min rest. The same subjects were reinvestigated during treatment with propranolol (Inderal® ICI England) used alone as therapy.

METHODS

The glucose tolerance tests (IVGTT) were performed in the morning after an overnight fast and with the subjects at rest. The IVGTT were performed before and during propranolol treatment.

The glucose stimulation was provided by *L.* ad administration of 0.5 g glucose/kg b.wt. as 50% solution injected during 2 min.

Serum insulin levels were measured in blood samples drawn at 0, 4, 6, 8 and 60 min after the beginning of the glucose injection. Insulin was determined with the Phadbas Insulin Test (Pharmacia Uppsala, Sweden) based upon the radioimmunoassay technique described by Wide et al. (6). The mean value of the insulin levels in serum at 4, 6 and 8 min and the difference between this value and the basal insulin level ($\Delta 4-8$ min) were calculated.

Blood samples for determination of glucose in plasma were taken at 10-min intervals during one hour for estimation of glucose tolerance, which was expressed as *K*-value calculated from the formula $K = (1 - 2 \times 100/T_2) \%$, where T_2 is the time (min) required for the concentration to be reduced by half its value.

RESULTS

The mean duration of treatment was 11 months (range 5-20) and the average daily propranolol dosage 510 mg (range 240-1000). No other drugs were given. There was no change in body weight between the two tests.

Mean values for fasting serum insulin before and during treatment with propranolol as well as the late insulin response to *i.v.* glucose expressed as serum insulin at 60 min after the glucose injection are shown in Table 1. The differences were not significant.

The early insulin response expressed as mean $\Delta 4-8$ min is also shown in Fig. 1. A wide distribution is seen, but there was no inhibition of the insulin response. The correlation between the two values in the same patient was low ($r = 0.35$) and not significant ($p > 0.05$).

There were no significant differences between the mean values of the two investigations with regard to glucose disappearance rates (Table 1).

Table 1 Serum insulin ($\mu\text{U/ml}$) levels and glucose disappearance rates in 10 men before and during propranolol treatment (mean \pm S.D.)

Insulin level	Before treatment	During treatment
Basal	13.7 \pm 6.0	14.2 \pm 5.3
$\Delta 4$ -6-8 min	71.5 \pm 30.5	66.9 \pm 32.1
60 min	41.9 \pm 33.6	49.1 \pm 22.0
K-value	1.68 \pm 0.78	1.42 \pm 0.48

DISCUSSION

The main purpose of antihypertensive treatment is to reduce the incidence of cardiovascular catastrophes. Hypertension is only one among many factors which promote the development of cardiovascular disease. Increased serum lipids and impaired carbohydrate tolerance are other such factors known from various epidemiological studies. It is essential that the treatment designed to influence one of these factors does not cause metabolic alterations which influence another in an unfavorable way. The reports by Cerami et al. (1, 3) that the initial insulin response to i.v. glucose is impaired after propranolol are alarming. This type of impairment in insulin response has been considered prediabetic (2). Thus the antihypertensive

effect of this drug could be counterbalanced by the changed insulin secretion pattern.

The proper means to study this effect on insulin secretion should be to compare the insulin response before and during long-term oral medication of propranolol in hypertensives. However, all previous studies with regard to the release of insulin have been performed as short-term tests after parenterally given propranolol. What is clinically important with regard to the overall preventive aspect of this effective antihypertensive drug is its influence on carbohydrate and insulin metabolism during long-term oral treatment.

The present study has not shown any alteration in glucose tolerance and insulin response to i.v. glucose during treatment with massive oral dosage

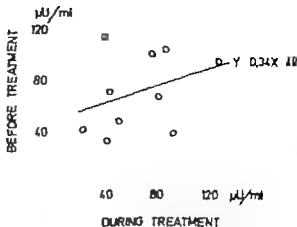


Fig. 1 Early insulin response ($\Delta 4$ -8 min) to i.v. glucose before and during long-term treatment with propranolol.

of propranolol. This is in accordance with a recent report (5) that the insulin secretion is independent of β -adrenergic receptors.

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PREHOSPITAL PHASE OF PATIENTS ADMITTED TO A CORONARY CARE UNIT

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Abstract The interval between onset of chest pain and admission to Stockholm Coronary Care Unit (CCU) has been investigated in 137 patients, 42% of whom were in the CCU within 3 hours (median about 3 1/2) of onset of symptoms. The main part of the interval was patient delay 30% calling for help within 30 min, yet as many waiting for 3 hours or more before doing so. Of those seeking help within 30 min 85% were in the CCU within 3 hours of onset of symptoms. Almost half the patients sought help by calling the Central Health Information Centre and 84% of patients were sent to hospital without first being seen by a physician. The subsequent diagnosis, i.e. acute myocardial infarction or not, did not affect the delay. Factors in the past medical history associated with shorter delay were previous CCU care and "visit to physician during last year. Delay was also shorter for patients not at home at the time of onset of symptoms and for those who encountered a positive attitude when calling for medical help. For unknown reasons there were significantly more patients with anterior infarcts among those with short delay. Sex, age and self-diagnosis did not affect the delay.

Most patients who die of an acute myocardial infarction (AMI) do so outside a hospital (1, 3, 9). Therefore several groups of workers have analysed the time elapsing between onset of symptoms of an AMI and admission to hospital (2, 3, 5, 6, 7, 8, 11, 15, 16, 19). This prehospital phase or "delay" poses a major challenge and raises numerous questions as to possible therapeutic approach as well as feasibility of reducing its length. As both geographic and organizational differences must be taken into account when studying this phase we thought it worthwhile to evaluate admission delay in Stockholm in order to find out which factors affect it.

Delay is commonly subdivided into 1) patient delay or "decision time", 2) doctor's delay, 3) transport delay and 4) hospital delay. Amongst

others Tyoe and Luma (19) have stated that the major part of the delay can be attributed to the "decision time", i.e. the interval between onset of symptoms and the patient's decision to seek medical help. These subdivisions of total delay no doubt serve their purpose. However, we feel that the patient, his personality, his powers to express himself and his knowledge of his ailment can affect all parts of the delay period. Total delay as well as its subsections have therefore been analysed.

METHODS

Serafimerlasarettet, with 300 medical beds and 7-bed CCU serves defined part of Greater Stockholm. Organization, admission and diagnostic criteria for the CCU have been given elsewhere (18).

For purposes of this study only consecutive patients with central chest pain as the dominant symptom have been included. Further criteria were: capability of giving a coherent history, survival after admission until an interview with one of the authors within 24 hours of admission, and onset of symptoms outside the hospital. Patients with longer delay than 48 hours are not admitted to the CCU. The material was collected during the autumn of 1971. Patients who following the ECG and routine enzyme tests, received the diagnosis of an AMI will be referred to as AMI patients and those who did not non-AMI patients.

In Stockholm patients who feel suddenly and severely ill do not in most cases contact a general practitioner but rather the Central Health Information Centre, with an easily remembered telephone number 90 000 which also covers other emergency services like ambulance, fire brigade and police. This central office is staffed by nurses and sends emergency calls by radio to chauffeur-driven doctors. If clear history of central chest pain is given the nurses will immediately send an ambulance to the patient for direct transport to hospital. The patients are brought to the hospital serving the district where they are domiciled. A salient feature in this study is also that considerable proportion of our admissions are not first admissions, i.e. patients there

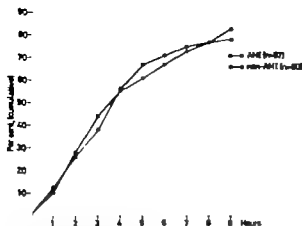


Fig. 1 Delay in admission expressed as admissions per hour (cumulative percentage) during first 9 hours from onset of chest pain

fore should know what to do where to go, and they often first phone the CCU to ask for advice.

A point which needs to be answered is what constitutes a "reasonable delay in this community. Considering the time taken as regards the effect of nitroglycerine telephone call for medical help ambulance to the patient transport to hospital the time necessary for a preliminary appraisal in the Casualty Department and transport to the CCU a delay of 2 hours is adequate and of 3 hours perhaps reasonable whereas longer delays strongly suggest a major interfering mechanism. Patients who had arrived within 3 hours of onset symptoms, i.e. "short delay patients" will in the sequel be referred to as SDPs and those with longer delay as LDPs.

MATERIAL

After exclusion of 7 patients with an equivocal diagnosis and 5 who died before an interview could be arranged there remained 137 patients, 57 with a subsequent diagnosis of AMI and 80 in whom this diagnosis could not be proven, i.e. non-AMI patients. On the other hand 64% of the latter had a history of ischaemic heart disease (IHD). The age and sex distribution is shown in Table I. Three of the AMI and one of the non-AMI patients died during hospitalization.

Table I Age and sex distribution of the 137 patients investigated

	AMI (n=57)	Non-AMI (n=80)
Age (y)		
Mean	65.5	63.3
Range	50-92	34-81
Sex		
Men	43	52
Women	14	28
Ratio	3:1	1.9

RESULTS

General findings The delay periods of the AMI and non-AMI patients are given in Fig. 1 showing that the two groups behaved similarly in this respect. Adding the two groups together the following figures emerge: 27% were admitted within 2 hours, 42% within 3 hours and well over half the patients (56%) were in the unit within the 4th hour after onset of chest pain.

Decision time (patient delay) (Table II). Forty patients (29%) 20 (35%) AMI and 20 (25%) non-AMI or someone in their surroundings acted within 1 hour by demanding medical aid in some form (ambulance, doctor or nurse). Not surprisingly this type of rapid action was associated with a high 3-hour (SDP) admission rate of 85%. About half of the patients delayed seeking medical help by 1 1/2 hours or more after onset of symptoms. The mechanism of patient delay was investigated in the 71% with a decision time exceeding 30 min. Of these patients 33% thought that their delay was due to a "thought pain would pass" mentality, 11% thought their symptoms not serious or just due to another bout of their angina, and 7% thought the pain was extracardiac in origin. Only 7% did not want to disturb and 1% felt too paralysed to call for help. Vague reasons not referable to any of the above were given by 12%.

Table II Patient delay (decision time) of the 137 patients before admission to CCU

Patients	Help called within					
	0-15'	16-30'	31-60'	61-120'	121-180'	≥3 hours
AMI	7	13	6	9	5	17
Non-AMI	9	11	9	15	10	26
All (cumulative percentage)	12	29	40	58	69	100

Table III *Attitude to call for help encountered from first contacted medical personnel*

Attitude encountered	Patients	
	No.	%
Type 1	76	33
Type 2	5	4
Type 3	30	22
Type 4	26	19

No differences in these respects were found between AMI and non-AMI patients

The person who called for medical help was in 36 instances (26%) the patient himself. It was observed that 31 (39%) of the non-AMI called for help themselves as compared to only 5 (10%) of the AMI patients ($p < 0.001$)

Pain relief was resorted to by 67% of the patients. 36% took nitroglycerine, 1% alcohol, 9% salicylates, 7% inappropriate drugs, e.g. digitals and dicoumarol and 15% combinations of the above. SDPs and LDPs and also AMI and non-AMI patients, when compared did not differ from each other in these respects

Medical help was sought by ringing the Central Health Office in 44% of the cases, their own physician in 12%, a nurse in 4% whereas 16% went directly to hospital with the help of a relative or friend or occasionally on their own. Direct contact with the hospital (usually the CCU) was taken by 24%. None of these primary medical contacts were related to any specific pattern as regards delay, nor did any method differ from the others as regards relative numbers of SDPs.

Attitude of first medical personnel contacted

Table IV *Interval as estimated by the patients between call for transport and departure for hospital*

Time	No. of pts.	Cumulative percentage
0-15 min	44	32
16-30 min	39	61
31-60 min	17	73
1-2 h	10	80
2-4 h	6	83
>4 h	4	88
Uncertain (own car or other form of transport)	17	(12)
Total	137	100

Table V *Hospital delay between Casualty Department and CCU*

Delay (min)	Patients		All (cumulative percentage)
	AMI	Non-AMI	
0-15	30	31	45
16-30	11	23	69
31-60	6	15	85
61-120	7	6	94
>120	2	4	99
Uncertain	1	1	100
Total	57	80	100

The advice given by the medical personnel first contacted could be classified into 1) advice or even help to obtain an ambulance immediately for transport to hospital, 2) similar advice but suggesting taxi or car, 3) delaying, e.g. arranging for a physician to come to the patient first or asking the patient to wait to see whether pain improves, and 4) unknown. As similar attitudes were encountered by both the AMI and non-AMI patients, they are accounted for together (Table III). Not surprisingly the most salutary type of attitude encountered (type 1) does affect delay as 67% of the 57 SDPs met this attitude as compared to 48% of LDPs ($p < 0.05$). Accordingly a delaying attitude (type 3) was met by 31% of LDPs as compared to 14% of SDPs ($p < 0.05$). A doctor's delay was present in 16%.

Interval between call for medical aid and departure for hospital. Table IV shows that 61% of the patients both AMI and non-AMI thought that they had obtained transport within 1 hour of request. Ambulance transport was employed by 83 (61%) and taxi or private car by 40 (29%) of the patients. Exact time estimates for the transport to hospital were not available.

Delay in hospital between Casualty Department and CCU. The time elapsing between arrival in hospital and in the CCU is presented in Table V. Altogether 45% of the patients had a shorter hospital delay than 15 min. Reasons for prolonged stay in the Casualty Department were investigated. Age was found not to matter, but final diagnosis did. Of the AMI patients 60% were in the CCU within 15 min of arrival in the hospital against 36% of the non-AMI patients ($p < 0.01$).

Previous medical history in relation to total delay. Angina pectoris had occurred in 83 (61%)

of the patients equally represented in SDPs (60%) and LDPs (61%). Similarly a previous AMI was of no significance. 37% of SDPs and 31% of LDPs gave a history of previous infarctions. Hypertension was reported by 35% of SDPs and 30% of LDPs and the corresponding figures for a history of congestive heart failure were 32% and 36% respectively. Also diabetes mellitus occurred about equally in the two groups—5 and 6% respectively.

The incidence of previous CCU care in the two groups was 37% of SDPs and 25% of LDPs (N.S.). However it was found that if a 2-hour interval is chosen for definition of short delay then previous CCU care does affect delay. Of 41 patients with a history of CCU care 20 (49%) had arrived in the CCU within 2 hours against 23 (24%) of the 96 patients with no previous CCU care ($p < 0.01$).

Delay was also related to visits to a physician during the last year. A majority of patients had in fact been to see a physician during the last year at least once. 91% of SDPs and 78% of LDPs ($p < 0.05$). Regular visits were reported by 39% of SDPs and 30% of LDPs (N.S.). Medical visits because of IHD per se were reported by 58% of SDPs and 48% of LDPs (N.S.).

Personal factors. Psychological factors and those related to education will be published elsewhere (10). Age, sex and marital status did not affect delay times significantly. Similarly heredity factors seemed non-significant—37% of SDPs had a heredity of IHD against 39% of the LDPs. Nor did awareness of myocardial infarction as measured by known contacts with a person with this disease during the last year (fellow patients in the CCU excepted) differ in the two groups. Of the SDPs 21% were aware of contacts with a person with AMI and 13% of the LDPs.

The relationship between delay and how patients perceived their own diagnosis was also investigated. A cardiac self-diagnosis was made by 65% of the patients. No difference in this respect was found between SDPs and LDPs (68% and 63%, respectively). Nor did a self-diagnosis of AMI affect delay—47% of SDPs thought they had an AMI as compared to 31% of LDPs. This difference falls just short of statistical significance. It should be remembered that these figures were obtained in a CCU—surroundings which well might affect a patient's diagnostic considerations.

External factors. Physical activity at onset does

not appear to influence delay. 39% of SDPs were asleep or resting against 51% of LDPs (N.S.). Being at home was more common (81%) among the LDPs than among the SDPs (65%) ($p < 0.05$). This difference was essentially due to the non-AMI LDPs, nearly all of whom (91%) had been at home. Those with a subsequent diagnosis of an AMI did not differ from the SDPs in this respect, 69% having been at home at the time of onset of symptoms.

Delay times were not related to weekdays or holidays. Among the SDPs 60% reported their onset of symptoms to have begun between 8.00 a.m. and 4.00 p.m. but this does not differ from 48% for the LDPs. The presence of a spouse or other person at the time of onset of symptoms did not seem to affect delay times. Thirty-eight per cent of SDPs and 34% of LDPs were alone at the time of onset of symptoms (N.S.).

Relationship between clinical features and delay. Anxiety did not seem to affect delay. In fact it was more often reported by the LDPs (49% against 35% N.S.). Nor did the type of pain (pain burning sensation oppression etc.) seem to matter and the same was found concerning radiation of pain—67% of SDPs and 75% of LDPs mentioning this feature. Associated autonomic symptoms also failed to shorten delay—75% of SDPs and 81% of LDPs reported symptoms including cold sweat, frozenness, nausea or vomiting. Arrhythmic sensations occurred in 43% of LDPs and 33% of SDPs (N.S.). Dyspnoea would appear to be an alarming symptom but was reported by 42% of SDPs and 45% of LDPs. Thus LDPs seemed to be subject to a slightly more marked symptomatology rather than the other way round.

More objective parameters of the acute condition also failed to differentiate between SDPs and LDPs among which admission systolic BP, heart rate and findings of congestive heart failure. That the ultimate diagnosis did not affect delay has been mentioned above. Furthermore infarction size (AMI patients only) as estimated by SGOT maximum level was similar in SDPs and LDPs. Anterior infarction occurred in 45% (10/22) of SDPs with AMI as compared to 17% (6/35) of LDPs ($p < 0.05$).

DISCUSSION

To summarize it seems that about half the patients were in the CCU within 3 hours of

onset of chest pain. These findings correspond to those in several other communities (7 13 15 16), whereas longer delays have also been reported (1 3 8 11). When comparing this study with similar foreign studies, it should be remembered that in the Stockholm area patients with central chest pain are usually sent direct to hospital and doctors' delay is often avoided. Thus Armstrong *et al.* (1) point to a considerably shorter delay for those who had not been referred via a general practitioner as compared to those who were.

In this evaluation the onset of symptoms has been equated with onset of myocardial necrosis. However several investigations have shown a marked discrepancy between autopsy findings and duration of the terminal attack (12, 14 20). An improved understanding of the earliest phases of an AMI which perhaps in part are characterized by "prodromal symptoms" may in the future open the way to a better definition of the true total delay in dealing with this disease thereby allowing for quicker intervention.

The dominant portion of the delay is the "decision time" (1 8 11 19). Like Sandler and Pistravos (13) we found that about one third of the patients call for medical assistance within 30 min. Our results are also similar to those of Armstrong *et al.* (1) and of G  christ (4) whereas longer decision times are given by others (8 11). Moss *et al.* (11) give age as one factor affecting the decision time, a finding not demonstrable in the present study.

Hospital delay figures have previously been reported by Tj  e and Luria (19). It is clear from the present study that, in spite of instructions to the Casualty Department staff, 30% of patients remained there for 30 min or more. On the other hand means of resuscitation do exist in the Casualty Department, reducing the importance of this time loss during which treatment is commonly started. It was found that a final diagnosis of AMI was related to a more rapid transfer to the CCU. Clearly special efforts are made by the staff in cases in which an AMI seems likely.

Generally the present findings appear disappointing. The factors found to affect total delay include: not at home, previous CCU care, anterior location of the infarction and visit to physician during the last year. With the exception of the latter, these findings are of limited practical

use. It is perhaps more interesting that patients with a history of diabetes, hypertension, angina and myocardial infarction are not overrepresented among those with shorter delay. These patients are easily available for education. It must be borne in mind, though, that it has not been shown that education shortens delay. Improved knowledge may strengthen a protective neurotic process of "denial" and thereby produce the opposite of what is intended. Denial as well as other factors involved in the delay process have recently been discussed by Yu (21) who also mentions the possible salutary role of family members and friends in reducing delay. The significant role of a second person, especially strangers in imitating the decision to seek help has also been pointed out by Hackett and Cassam (7).

Of more practical use is the finding, though not surprising, that a positive attitude on the part of the first medical personnel contacted does seem to affect total delay. Failure to arrange for immediate ambulance transport clearly lengthens the total delay. It must be concluded that in this type of patients with central chest pain the response of the medical personnel first contacted should be to organize rapid admission to a CCU.

Other delay lengthening factors include week ends and daytime (11), time of day (1 — 5), preceding crescendo angina (15) and a history of cardiac disease or misinterpretation of symptoms as being a recurrence of angina (6). There was no indication in the present study that any of these factors had a significant influence. Nor did Tj  e and Luria (19) consider that a history of AMI affected delay. They also found that a self-diagnosis of AMI does not shorten delay which is in accordance with the present study. This finding again raises doubts as to the potential efficacy of educational measures which would be expected to provide information enabling patients to diagnose their condition. Yet a self-diagnosis of AMI apparently does not shorten the delay significantly, perhaps again suggesting "denial" mechanism. Alternatively although capable of diagnosing a possible AMI patients are genuinely aware of the severe threat to their lives during the early phases of this condition.

A factor found to be unrelated to delay was the severity of the acute attack as measured by the Peel score, whereas subjective assessment is related to delay (7). Recently Barber *et al.* (2) found

a relationship between severity of attack and patient delay. In the present study severity was not measured per se but different symptoms and clinical findings associated with an AMI played no significant role in influencing delay. A curious finding was an overrepresentation of anterior infarctions amongst SDPs. In this context it is of interest to note that differing symptomatology has been reported when comparing anterior infarctions with those of other localization (17).

As in other similar studies the present investigation was based on information gathered from the survivors of the most crucial early period and some caution must be exercised when transferring the results to those who die early never reaching a hospital. This latter group is of interest in evaluating early phases of an acute manifestation of IHD. Relevant figures in the Stockholm area are given in Wiklund's monograph (20) where a very high mortality rate within the first 15 min (61% for males and 48% for females) is reported. These patients may be considered beyond medical reach with present means except in special circumstances where there may be resuscitation facilities i.e. industrial plants, sport stadiums etc. A poor yield remains likely as some 70% of cases report onset of symptoms in their homes as was found in this study as well as by others (9).

A feasible aim may perhaps be to have the majority of patients in this community in a CCU within 2 hours of onset of symptoms. This is not impossible as is shown by the 27% of our patients who do arrive within this time. With this goal in mind, and with reference to the figures given by Wiklund (20) and Kuller et al. (9) it might be possible to bring about 25% of those who at present die medically unattended outside hospitals, to proper care. This figure however presumes that these patients have a recognizable symptomatology.

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LATE RISE IN SGPT AFTER ACUTE MYOCARDIAL INFARCTION

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Abstract The present study draws attention to late rise in SGPT following acute myocardial infarction (AMI) beginning about 4 days after the SGOT max. This observation is illustrated by an investigation of 7 patients, selected because of this finding, and 7 controls, also with an AMI but without this enzyme pattern. Peak SGOT and LDH enzyme levels during the acute phase were similar in the two groups. The late SGPT rise was associated with high serum alkaline phosphatases 2 weeks after the AMI but not 5-9 months later. Two weeks after onset of symptoms BSP excretion was commonly impaired but failed to separate the two groups. In contrast to the controls, BSP excretion was significantly improved at reinvestigation 5-9 months after the AMI in the group with the SGPT rise.

Impaired hepatic function in patients with chronic congestive heart failure (CHF) is well recognized (4, 8, 11, 13, 16, 19, 22, 4, 28, 29) but little attention has been paid to this in AMI patients (1, 18). Concomitant with a diminished excretory capacity for bromsulphalein (BSP) there is often also other laboratory evidence of hepatic impairment in chronic CHF (3, 11, 13, 14, 16, 22, 24, 28, 29, 30). Changes in liver perfusion, anoxia, raised venous pressure as well as malnutrition have been advanced as possible causal mechanisms (11, 19, 23, 28).

In uncomplicated AMI rises in serum alanine transaminase (SGPT) roughly follow the rises in serum aspartate aminotransferase (SGOT) but do not increase above the upper normal level unless the infarction is extensive. Sources other than the heart should therefore be suspected in cases with unexpected SGPT rises in the early phase (4, 30). Excessively high levels—more often seen when severe right-sided heart failure, shock or pulmonary embolism complicate myocardial infarction—are thought to indicate liver cell injury (3,

17) and can at times jeopardise the serum enzyme diagnosis of AMI (4, 5).

The present investigation of patients with AMI was initiated by repeated observations of a late rise in SGPT beginning about a week after onset of symptoms and exceeding simultaneous SGOT values. This rise was usually not accompanied by a rise in SGOT and was therefore assumed not to reflect reinfarction, nor was there any clinical evidence of this being the case. In an attempt to clarify this enzyme rise a further evaluation was made of hepatic function in 14 patients with AMI, 7 with and 7 without this enzyme rise.

METHODS

The patients were treated in the Coronary Care Unit (CCU), Serafimerlasarettet. The criteria for admission and diagnosis, policy of treatment etc. have been given elsewhere (25). SGOT, SGPT, LDH and LDH (the least stable portion of LDH) were analysed routinely on admission and every 12 hours while the patients were in the CCU and twice a week for the remainder of the hospital stay and were repeated at the time of the liver function studies. Analyses were made with *Reactions Rate Analyzer (LKB 3650)* connected to an evaluation unit (*Optilab, Bo Philip Instrumentation, Stockholm*) (6, 26). Normal upper levels for SGOT, SGPT and serum alkaline phosphatase (AP) were ≤ 40 mU/ml and for LDH ≤ 350 mU/ml. A standard BSP excretion test (5 mg/kg b.w.t.) was performed about two weeks after onset of the AMI and again 5-9 months later. A retention of 5% or more after 45 min was considered pathological. At the time of reinvestigation the patients were also questioned regarding their alcohol consumption.

The definition of this late secondary rise in SGPT after AMI for the present study was a rise in the enzyme exceeding simultaneous SGOT levels beginning 4-5 days after SGOT maximum. This implies that the enzyme rise starts about one week after onset of symptoms and that the SGPT need not rise to pathological levels.

Table I *Clinical findings in the study group and the controls*

Ap=angina pectoris, Db=diabetes, Ht=hypertension, MI=myocardial infarction

Case no.	Age (y)	Sex	History of					Infarction site	Heart size (ml/m ² /BSA)	
			CHF	Ap	Db	Ht	MI			
Study group										
1	64	♂	-	-	-	-	-	Inferolateral	540	
2	71	♂	-	-	-	-	-	Inferolateral	710	
3	55	♂	-	+	-	-	-	Inferolateral	370	
4	66	♂	-	+	-	-	-	Inferior	430	
5	64	♂	+	+	-	-	+	Inferior	370	
6	73	♂	-	+	-	-	+	Inferior	480	
7	68	♂	-	-	-	-	-	Inferior	380	
Control group										
1	81	♀	-	+	-	+	-	Anterolateral	310	
2	60	♂	-	-	+	-	-	Inferior	760	
3	56	♂	-	+	-	-	-	Anterolateral	330	
4	71	♀	-	+	-	-	-	Inferolateral	380	
5	61	♀	-	+	-	+	-	Inferior	510	
6	61	♀	-	-	-	-	-	Inferior	350	
7	63	♀	-	-	+	-	-	Anterior	420	

PATIENTS

The present study presents the findings in 14 non-consecutive patient with transmural AMI 7 with a secondary SGPT rise (study group) and 7 without this enzyme pattern (control). Each time a patient with secondary SGPT rise was followed, a control—treated in the unit at the same time but without evidence of similar enzyme pattern—was selected. All patients were treated in the unit from May to Sept. 1972.

The clinical characteristics of the patients are given in Table I. The groups were similar as regards sex, age, history of heart failure, angina pectoris, diabetes and hypertension. Two patients in the study group but none of the controls had sustained a previous myocardial infarction. No history of liver disease or alcohol abuse was given.

During the early phase of the AMI 5 patients in the study group but none of the controls had ventricular dysrhythmias, wherefore the former received more anti-

Table II *Enzyme values in the study group and in the controls*

Case no.	SGOT max. (mU/ml)	SGPT at SGOT max. (mU/ml)	LDH max. (mU/ml)	LDH at LDH max. (%)	AP max. (mU/ml)	SGPT max. (secondary rise) (mU/ml)	SGOT at SGPT max. (mU/ml)
<i>Study group</i>							
1	195	33	1 030	90	68	81	55
2	205	40	1 000	84	35	83	49
3	179	47	1 300	87	54	123	100
4	253	51	1 340	87	28	87	59
5	112	31	625	78	44	80	42
6	65	21	410	81	36	35	26
7	62	20	420	76	26	33	21
<i>Control group</i>							
1	138	39	730	78	26	-	-
2	350	42	1 500	82	13	-	-
3	89	20	485	68	22	-	-
4	143	21	720	85	38	-	-
5	87	17	430	86	27	-	-
6	141	24	750	85	30	-	-
7	86	21	700	77	18	-	-
p*	N.S.	N.S.	N.S.	N.S.	<0.025		

Study group—control group.

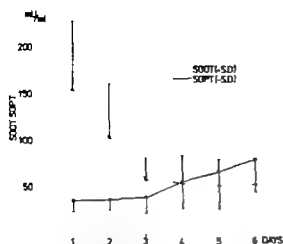


Fig 1 Enzyme pattern of SGOT and SGPT in 7 patients with secondary rise in SGPT Day 1 corresponding to recorded SGOT maximum.

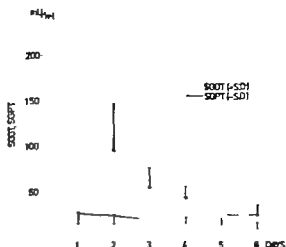


Fig 2 Enzyme pattern of SGOT and SGPT in 7 control patients. Day 1 corresponding to recorded SGOT maximum.

arrhythmic drugs, but the groups were similar with respect to other treatment including digitalis, diuretics, analgetics, sedatives and anticoagulants. CHF was noted in 6 patients of the study group and 14 of the controls. One patient in the control group was hypotensive and had also the highest AP value and the most markedly impaired BSP excretion. At discharge 3 patients in the study group and one of the controls were on digitalis and at the time of the reinvestigation the number had risen to 5 and 2, respectively. No patient had had reinfarction in the meantime.

All patients in the study group and 4 of the controls had ECG signs suggesting involvement of the inferior wall of the left ventricle. The mean heart size at discharge was 469 ml/m² BSA in the study group and 437 ml/m² BSA in the controls (N.S.).

RESULTS

Laboratory findings The serum enzyme levels are given in Table II. The mean maximum SGOT

Table III. Liver function tests and enzyme values (mU/ml) in the study group and in the controls

Case no.	Enzymes at first BSP test			First BSP retention after 45 min (%)	Enzymes at second BSP test			Second BSP retention after 45 min (%)
	SGPT	SGOT	AP		SGPT	SGOT	AP	
Study group								
1	22	20	46	18	13	15	20	7
2	20	37	26	16	19	14	12	8
3	88	48	33	6	12	16	13	4
4	53	21	28	13	18	21	19	7
5	40	20	31	7	17	20	1	7
6	15	13	30	16	13	21	37	12
7	14	18	22	10	15	17	14	8
Control group								
1	17	34	15	25	10	18	14	12
2	12	15	12	7	11	20	12	6
3	26	29	17	6	16	18	22	5
4	12	14	38	8	13	18	22	8
5	13	16	27	8	10	14	16	8
6	10	13	31	3	10	15	22	3
7	13	18	17	3	11	16	22	
P	<0.025	N.S.	<0.05	N.S.	<0.01	N.S.	N.S.	N.S.

Study group—control group.

In the study group was 153 mU/ml and in the controls 148 mU/ml. The mean SGPT in the study group at the time of maximum SGOT was insignificantly higher 35 mU/ml as compared to 26 mU/ml in the controls. The secondary SGPT rise exceeded the normal level in 5 of the study group patients being within the normal range in 2. The enzyme patterns are shown in Figs 1 and 2.

The SGOT, SGPT and AP values at the time of the first BSP test performed 10-17 days after the AMI are given in Table III. The groups still differ in SGPT. Also AP levels were significantly higher in the study group ($p < 0.05$) but the difference in BSP retention was not (means 12.3% and 8.6% respectively). All patients except two of the controls had abnormal BSP retention.

At the time of the second BSP test 5-9 months after the AMI the SGPT values were within the normal range but still significantly higher ($p < 0.01$) in the study group but no differences were found in AP, SGOT or the BSP test. If liver function at the time of the first BSP test is compared to that at the time of the second no differences were found within the control group. In contrast AP levels decreased significantly ($p < 0.05$) and BSP excretion showed a significant improvement ($p < 0.02$) in the study group (Table III).

DISCUSSION

Using routine enzyme tests we identified a group of patients with a late rise in SGPT beginning towards the end of the first week after an AMI in contrast to the commonly observed early minor rise that roughly parallels the SGOT rise. To find out whether this late SGPT rise was associated with impaired hepatic function the latter was evaluated with standard liver tests including BSP loadings and AP determinations (3-19).

Hepatic function in AMI has been investigated previously. Aber et al (1) performed a modified BSP test in the earliest stages of an AMI and found impaired liver function which was related to the clinical condition of the patients. They supported the finding of Van der Helm et al (27) that the rise in LDH 5 isoenzyme activity originated from the liver since that enzyme pattern was associated with poor BSP excretion. However Albertini et al (2) proposed that an abnormal BSP test does not necessarily indicate liver cell damage but merely reflects circulatory failure.

The relationship between hepatic blood flow and abnormal liver function in chronic CHF remains to be delineated. Myers and Hickam (20) found that there was a decrease in hepatic blood flow in chronic CHF roughly proportional to the decrease in cardiac output. Somewhat at variance with this Losowsky et al (19) found rather normal hepatic blood flow in such patients even in the presence of severely impaired BSP excretory capacity. The findings vary and there are reports (11) of a parallel decrease in BSP excretion and severity of heart failure.

A positive correlation between right heart pressures and the BSP test has been noted (11, 19, 28). In patients with pulmonary insufficiency however no such simple relationship between right-sided pressures and liver damage seems to exist (21).

Collins et al (9) have pointed to an association between a raised central venous pressure, dysrhythmias and inferior infarction. This, perhaps has a bearing on the present study as all patients with ventricular dysrhythmias were in the study group all of whom also had inferior infarctions. More antiarrhythmic agents were therefore used in the study group a factor which cannot be excluded as a cause of liver damage. However different drugs were employed and they were given at varying stages of the illness.

In the study group the BSP values were significantly lower in the second than in the first test. The improvement of AP and BSP excretory capacity suggests improvement of hepatic function with time in these patients. Ewen and Griffiths (12) however have proposed that changes in some enzymes e.g. AP, could be due to necrosis as well as reparative processes. Felder et al (13) suggested that AP was one of the more sensitive tests in CHF but in contrast to the present findings White et al (29) state that AP levels are not corrected with time in patients with CHF. Furthermore Betro (7) described increases in AP in patients with AMI but without clinical evidence of heart failure and a hepatic source was assumed.

The study group did not differ from the controls as regards clinical signs of circulatory failure or history of CHF. Furthermore no differences were noted as regards venous pressure and infarction size as estimated by enzyme maximum. The latter may however be an inadequate measure as regards the relationship between myocardial damage and circulatory impairment. Infarction of papillary

muscles as well as right ventricular infarction may be of considerable circulatory significance. It is noteworthy that all patients with a rise in SGPT had inferior infarctions, and these infarctions are particularly prone to right ventricular extension (10).

The present study thus suggests that late rises in SGPT following an AMI are mainly characterized by associated elevated AP levels, whereas the significance of hepatic function remains to be determined.

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ELECTROCARDIOGRAPHIC ARTERIOGRAPHIC AND HEMODYNAMIC CORRELATION STUDIES IN CORONARY HEART PATIENTS WITH ANEURYSM OR AKINESIS OF LEFT VENTRICLE

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Abstract. The results from left ventricular cineangiography have been compared with ECG, coronary arteriographic and hemodynamic findings in 59 selected patients with coronary heart disease. All patients showed abnormal left ventricular contraction patterns; ventricular aneurysm was present in 22 patients, and ventricular akinesis in 37. All patients with aneurysm had previous history of myocardial infarction and there was close correlation between the ECG pattern and the angiographic findings, while unspecific ECGs were commonly seen in patients with akinesis. All patients had severe coronary artery stenoses. No aneurysm was seen without occlusion of the major local artery while the anatomical location of the arterial stenosis showed less consistent relationship to the angiocardigraphic findings in patients with akinesis. Left ventricular end-diastolic pressures were analyzed before and after left ventricular angiography and found to be elevated in most patients. No differences were found between patients with aneurysm compared with those with akinesis.

Cineangiography of the left ventricle together with selective cinecoronary arteriography provides valuable information about the functional state of the left ventricular wall and the anatomy of the coronary arteries. To-day these two methods have been accepted as useful tools in the evaluation of patients with coronary artery disease.

In certain patients with coronary artery disease one may differentiate between two separate ventriculographic patterns: ventricular aneurysm meaning a localized protrusion or angulation of a portion of the left ventricle visible beyond the surface (4) and ventricular akinesis meaning a local absence of ventricular contractions but without abnormal bulges or changes in contour (7). An

ordinary cardiac X-ray may occasionally be able to distinguish bulges or changes in contour characteristic of aneurysm but fails to identify an akinetic area which is noticeable only during active systole.

Attempts to define and differentiate these two separate ventriculographic patterns have been few. In particular there are few studies comparing ECG, arteriographic and hemodynamic correlation data in patients with ventricular aneurysm and in patients with ventricular akinesis.

MATERIAL AND METHODS

The study is based on the findings in 59 selected patients referred to our Medical Department for diagnostic evaluation of their coronary vessels and left ventricular function with the purpose of cardiac surgery. The patients were with few exceptions middle-aged men. A total of 43 patients had a proven history of myocardial infarction, while angina pectoris without known infarction was present in the remaining 16. None of them had overt cardiac failure.

Selection of the patients was based on the findings of left ventricular angiograms, which were taken in the right and anterior oblique projection. Only patients with anatomical or functional abnormalities accepted as diagnostic of left ventricular aneurysm or akinesis were included.

Based on the following criteria the patients were divided into four groups. *Aneurysm of anterior wall* (16 patients). Clear-cut saccular protrusion of the anterior wall, in many cases also including the apical region. Normal contour and contraction of the inferior wall and the constrictor plane (outflow tract to inferior wall). *Aneurysm of inferior wall* (5 patients). Clear-cut saccular protrusion of the inferior wall or the dia-

phragmatic region immediately adjacent to the mitral ring. Good contractions in the anterior wall and apical area. *Akinesis of anterior wall* (13 patients) Absence of contractions at the anterior wall and usually also involving the apical region. Good contractions in the diaphragmatic wall. *Akinesis of inferior wall* (74 patients) Absence of contractions in the diaphragmatic region immediately adjacent to the mitral ring and generally extending to the posterior papillary muscle. Good contractions in the anterior wall and apical area.

Selective cinecoronary angiography was carried out according to the technique described by Judkins (8). The coronary arteriograms were performed in left anterior and right anterior oblique and additional projections when needed. The main coronary arteries: the anterior descending branch and the circumflex branch of the left coronary artery and the right coronary artery and their tertiary branches were identified and evaluated separately. In regard to the left coronary artery none of the patients examined had a localized severe stenosis of the main stem. The structural changes observed were graded according to the classification presented by Malcomson et al. (12) giving each of the three main coronary arteries a score from 0 (no atherosclerotic changes visible) to 3 (total occlusion of the vessel). The three scores were added together to give a total score which represented the overall obstructive disease for these three arteries of the heart.

The contrast material used was Isopaque® Coronar (sotrizoate meglumine/Na/Ca (Sb/SH)) 370 mg/ml. For left ventricular angiography the single dose was 45 ml injected through a Formocath catheter furnished with small end hole and two 1.5 cm long side splits near to the tip. The piston pressure of the high pressure syringe was set at 6 kg/cm². For the selective coronary angiographies preshaped catheters (Ducor Cordis Corporation) were used and hand injections of 5-9 ml contrast medium were given repeatedly. Cinecoronography was performed with a 35 mm Arflex camera in combination with image intensifier running 75 frames/sec. For preliminary studies a video taperecorder (OD-440 Old Delft, Holland) was used.

Extrasystoles were frequently seen during injection of contrast medium in the left ventricle. The assessment of left ventricular contractions was made during stable rhythm.

Left ventricular diastolic pressure was measured before and after 2 min following left ventricular angiography. Pressures were recorded using Elema Schönmader recording equipment (Transducer EMT 35 and Mingograf 81). The baseline was at the mid-chest level. Left ventricular diastolic pressures were divided into early mid and late diastolic pressures. The late end-diastolic pressure was taken as occurring at the point where the left ventricular systolic wave interrupted the atrial transmitted "a" wave—i.e. 0.052 sec after the beginning of the Q wave (3).

A 12-lead ECG at rest was taken. Based on the ECG findings 18 patients had a normal QRS complex, while 41 had a pathological QRS complex indicating previous transmural myocardial infarction. The infarction was localized as anterior, inferior or both using the standard

Table 1 *Clinical diagnosis in 59 patients with coronary heart disease and akinesis or aneurysm of left ventricle*

Left ventricular angiographic findings	Clinical diagnosis	
	Angina pectoris	Status post-myocardial infarction
Anterior wall akinesis	6	7
Inferior wall akinesis	III	14
Anterior wall aneurysm		16
Inferior wall aneurysm		6
Total	16	43

criteria. A more detailed ECG localization was not attempted as the cases in each group were too few.

RESULTS

Clinical manifestation. Information concerning the previous clinical manifestation of coronary artery disease and the state of the left ventricular function obtained from the ventriculograms is summarized in Table I.

Of a total of 37 patients with akinesis 16 (44%) were without previous known infarction, while the remaining 21 patients in this group, as well as all 22 patients with aneurysm had also a documented previous history of myocardial infarction.

ECG findings. A normal or borderline ECG at rest was found in 8 of 13 patients with anterior wall akinesis and in 10 of 24 with inferior wall akinesis. Thus 18 (49%) of 37 patients with akinesis had a normal QRS complex. The remaining patients in this group had an abnormal QRS complex and the ECG pattern of an old myocardial infarction correlated well with the anatomical location of the akinesis. All patients with aneurysm had an abnormal QRS complex. In these patients a close correlation was noted when the location of the aneurysm was compared with the location of earlier infarction demonstrated in the ECG.

Distribution of coronary arterial disease. This series of patients has also been reviewed concerning the presence of single, double or triple vessel disease. With a few exceptions all patients had either double or more commonly triple vessel involvement as shown in Table II. In this respect there seemed to be no difference between

Table II *Relation between left ventricular angiographic findings and severity of coronary artery disease*

Left ventricular angiographic findings	Severity of coronary artery disease			
	Open	Single vessel	Double vessel	Triple vessel
Anterior wall akinesis (n=13)	1		6	6
Inferior wall akinesis (n=24)			6	18
Anterior wall aneurysm (n=16)			4	10
Inferior wall aneurysm (n=6)			1	5
Total	1	2	17	39

akinesis and aneurysm patients. Using the scale of coronary artery stenosis described the relationship between the cineventriculographic findings and the severity of coronary artery changes is shown in Fig. 1 (the small group of patients with inferior wall aneurysm is excluded). The highest percentage of coronary arteries free of atherosclerosis was found in patients with anterior wall akinesis. In this group subtotal or total obstruction of at least one coronary artery was found in slightly more than one half of the patients. Subtotal or total obstructions were more common in patients with inferior wall akinesis or anterior wall aneurysm and were found in approximately 70% of the arteries in these two groups. Both in extent and location the coronary artery lesions bore some relation to the angiographic localization, although the correlation was not a rigid one. This is shown

in Table III. The left anterior descending branch was occluded in all but one patient with anterior wall aneurysm. The left anterior descending branch was also the one most commonly involved in patients with anterior wall akinesis but was occluded in only 5 of 13 patients. The posterior descending branch of the right coronary artery or posterior medial branches from the circumflex artery were the most severely affected in 24 patients with inferior wall akinesis and were occluded in 15. The circumflex artery was about equally involved in the four categories of patients. The intergroup differences in total arterial score were not statistically significant.

Hemodynamic findings In 44 of the 59 patients pre and postangiographic left ventricular end-diastolic pressures were available. The preangiographic pressures were normal in 8 patients and elevated above upper normal range (10–12 mmHg) averaging 17 mmHg, in 36. There was no significant difference between mean left ventricular end-diastolic pressures in the three main groups (anterior and inferior wall akinesis, anterior wall aneurysm). The postangiographic left ventricular end-diastolic pressures were with a few exceptions higher than those recorded before angiography and rose to an average of 23 mmHg. The pressure rise was of approximately the same magnitude in the three main groups.

DISCUSSION

The relation between coronary artery disease and degree and location of myocardial damage is complicated as is natural due to the complexity

Table III. *Coronary artery changes in 59 patients with akinesis or aneurysm of left ventricle*

LAD=anterior descending branch left coronary artery CIRC=circumflex branch left coronary artery POST=posterior right coronary artery

Classification of coronary artery disease: grade 0 to 3

Left ventricular angiographic findings	Coronary arterial scores (mean with range)				
	LAD	CIRC	POST	LAD+CIRC	LAD+CIRC+POST
Anterior wall akinesis (n=13)	1.9 (0–3)	1 (0–2)	1.5 (0–3)	3.1 (0–3)	4.7 (0–3)
Inferior wall akinesis (n=24)	1.6 (0–3)	2.0 (0–3)	2.5 (0–3)	3.6 (0–3)	6.1 (0–3)
Anterior wall aneurysm (n=16)	2.9 (2–3)	1.4 (0–3)	1.4 (0–3)	4.2 (0–3)	5.7 (0–3)
Inferior wall aneurysm (n=6)	2.3 (1–3)	1.5 (0–3)	2.1 (1–3)	3.8 (0–3)	5.9 (0–3)

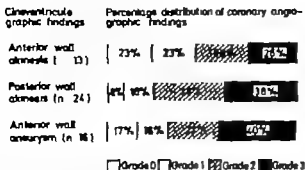


Fig. 1 Severity of coronary artery disease. Analysis of 53 patients with akinesis or aneurysm of left ventricle. The right coronary artery, left anterior descending branch and left circumflex branch are considered. Structural changes graded according to the classification by Malmberg et al. (12)

of collaterals and anastomoses. In the present study left ventricular aneurysms were always associated with gross coronary artery disease involving either stenosis or obstructions of the coronary artery supplying the area of the left ventricle where the aneurysm was found. The striking predominance of left anterior descending coronary artery involvement with associated anterior or apical aneurysm is in accordance with other studies (13). Most patients with akinesis also displayed marked and diffuse coronary involvement but, contrary to the aneurysm, the akinesis did not occur as predicted by the angiographic pattern of the coronary arterial disease.

Animal experiments have shown that the extent and severity of the myocardial lesion depend directly on the degree and localization of the coronary occlusion (11). In man this seems to be true in aneurysm, but is a less consistent finding in coronary patients with akinesis. Although some authors claim that in akinesis there is a very good correspondence between the site of vessel disease and zone of abnormal contractility (5) this was not true either in our series or in a similar study published by Björk et al. (2).

All patients with aneurysm showed ECG evidence of old myocardial infarction. Interestingly however no evidence of ECG abnormalities at rest were found in approximately 50% of patients with akinesis. Several explanations may be offered in this connection. It is possible that the resting ECG often fails to reveal a small infarct manifested in ECG as a left ventricular akinesis as suggested by Lichtén (10). Another possibility is that the muscle zone which fails to contract nor-

mally has not yet infarcted due to prolonged ischemia. Repeated sublethal ischemia may destroy sensitive structures essential to excitation-contraction coupling and spare the contractile protein matrix itself (7). The possibility that the akinesis occurs as a result of injection of contrast medium into the left ventricle cannot be excluded, but is improbable as this effect can only be observed 6–10 sec after injection (1).

The observation that development of an aneurysm is critical from a hemodynamic point of view is well documented (7–9). According to Klein et al. (9) the involvement of more than 20% of the ventricular surface by non-contractile tissue will result in an increase in left ventricular end-diastolic pressure while the size of an akinetic area assumed to be critical from a hemodynamic point of view is uncertain.

In our patients the left ventricular end-diastolic pressure was abnormal in the great majority of patients in whom this parameter was measured and the difference between left ventricular end-diastolic pressures in patients with akinesis compared with those with aneurysm was small and insignificant. The rise in end-diastolic pressure after intracardiac injection of radiographic contrast material represents a stress test of left ventricular performance (6). The postangiographic pressures were of about the same magnitude in the two groups of patients. Taken together our observations seem to indicate that aknetic areas of the left ventricle have a similar effect on left ventricular end-diastolic pressure as aneurysm. The statement that left ventricular akinesis in itself may cause heart failure however remains to be proved and needs more extensive investigations.

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SERUM LEVELS AND RENAL EXCRETION OF DIGOXIN IN THE ELDERLY

A Comparison Between Three Different Preparations

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Abstract Bioavailability of digoxin from three different preparations (S, W and L) has been studied by radioimmunoassay of serum and urinary digoxin in a cross-over study. In 25 elderly patients preparations W and L caused significantly higher serum digoxin levels than S, but did not differ from each other in the rapidity of the change or the levels reached. Serum and urinary digoxin of 11 patients were measured on two consecutive days, 10-14 days after changing the preparation. The daily urinary excretion of digoxin is a good criterion of absorption and seems to be in certain balance with digoxin in the serum. Creatinine and digoxin clearances were positively correlated. In spite of the relatively low creatinine clearances of these elderly patients the better absorption of preparations W and L was greatly compensated by increased renal excretion of digoxin. The dosage of digoxin, when changing preparations, should be adjusted individually.

Comparisons based on acute absorption tests, often with healthy subjects have indicated differences between various digoxin preparations (17). There are, however, various opinions about the clinical significance of these differences in bioavailability. Clinically the most important changes are those which take place during maintenance therapy when a preparation is being replaced by another. Manninen et al. (18) showed that when they replaced one preparation by another therapeutically significant changes took place.

Digoxin is eliminated mainly unchanged by renal excretion. It is therefore possible that during maintenance therapy the 24-hour urinary excretion of digoxin will reflect more reliably the total amount of digoxin absorbed than does the concentration in the serum. We have therefore compared three digoxin preparations, administered to the same group of patients during maintenance

therapy by measuring the concentration of digoxin both in the serum and in the urine. The group we chose consisted of aged patients expected to be most susceptible to any changes that may take place.

MATERIAL AND METHOD

The study was carried out in two stages on patients suffering from heart insufficiency in Turku City Hospital. The preparations compared were Digoxin (S) by Star used as basic drug for all the patients, Lanoxin Novum (W) by Wellcome, and Lanadox Novum (L) by Leiras (not yet launched on to the market) used as test preparation.

Stage 1 The rapidity of the change of the steady state serum digoxin level was studied in 25 patients (19 female and 6 male) of average age 70 years. All three preparations were compared in a cross-over manner on 13 patients, preparation S was compared only with one of the preparations W or L on 12 patients.

Digoxin was given orally once a day either 0.250 or 0.375 mg, and no change in dosage was made during the study. The samples were collected 24 hours after the previous dose. A steady state concentration was determined on two consecutive days in patients taking preparation S and 1-2 and 10-14 days after the change of preparation. Before changing over to the third preparation, the patients were again given preparation S for fortnight.

Stage 2 was carried out on 11 aged patients (9 female and 2 male) with heart insufficiency. Their average age was 77 years (range 65-84). The serum digoxin levels of all the patients were compared after 10-14 days therapy on two consecutive days with each preparation. As in stage 1 serum sampling was carried out 24 hours after the previous digoxin dose. On the same days 24-hour urine was collected with indwelling catheter. On the test day an ECG was taken and the digoxin clearance was calculated by analysing the concentrations of 4-hour urine and of serum digoxin on the same day. Digoxin clearance was

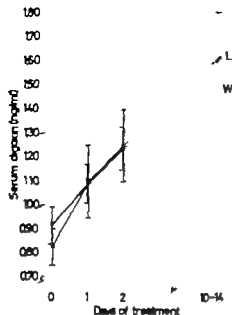


Fig. 1 Change of serum digoxin concentrations (\pm S.E.M.) after replacing preparation S (at 0) by L or W.

173 m^2 BSA. The 10-14 days therapy with different preparations was carried out by following the order S-W-S-L or S-L-S-W. The latter part of the study followed the principles of stage 1. Simultaneous use of diuretics or other drugs during the study was not restricted.

Assay of digoxin
 Immunological determination of digoxin in the serum and in the urine was always carried out in duplicate by using the method of Smith et al. (20). Digoxin was extracted twice from the urine with dichloromethane evaporated to dryness and diluted into suitable amount of phosphate buffer solution for determination. The mean recovery of the method was 89%. The antiserum was prepared according to the method of Smith et al. (21) by immunizing rabbits with digoxin-protein conjugate.

RESULTS

In stage 1 we observed that the serum digoxin levels increased clearly when changing preparation S to either W or L (Fig. 1). The rapidity of this change was the same for both brands although individual differences were relatively great.

The mean steady state serum digoxin level (\pm S.E.M.) was 0.92 ± 0.08 before changing preparation S to W. When S was changed to W respectively L, the corresponding figures were 1.48 ± 0.12 and 0.83 ± 0.06 . Both changes were statistically highly significant ($p < 0.001$).

The material and results of stage 2 are presented

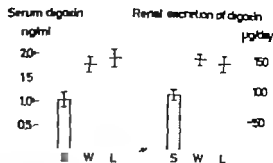


Fig. 2 Serum and 24-hour urinary digoxin levels (mean \pm S.E.M.) in 11 patients treated with preparations S, W and L.

In Table 1 Preparations W and L caused an increase both in the mean steady state serum levels and in the urinary excretion of digoxin when compared to preparation S. These changes were all statistically highly significant ($p < 0.001$). The mean levels are illustrated in Fig. 2. It is seen that when a higher serum level is achieved there is an almost equal increase in the mean urinary excretion of digoxin. In one patient (no. 8) however no increase in the serum digoxin levels was found when changing to the preparations with better absorption. The good absorption was totally compensated by an increase in the renal excretion of digoxin. The creatinine clearance of this patient was not reduced.

Creatinine clearance was somewhat decreased in several of these elderly patients. There appears to be a positive correlation between creatinine and digoxin clearances (Fig. 3). The measurements

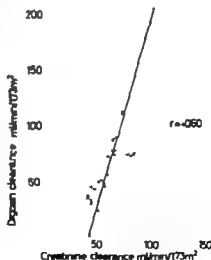


Fig. 3 Correlation between creatinine and digoxin clearances in 11 patients during maintenance therapy.

Table I *Steady state serum digoxin levels and daily urinary excretion of digoxin of 11 elderly patients*

Pat. no.	Age (y)	Sex	Average creatinine clearance (ml/min/1.73 m ²)	Daily digoxin dose		Average steady state serum digoxin (ng/ml)			Average urinary digoxin excretion (µg/d.)			Average digoxin excretion (% of daily dose)			
				(µg/d.)	(µg/kg)	S	W	L	S	W	L	S	W	L	
1	70	♂	74	250	5.6	0.45	1.8	1.75	42.8	159.6	1.5	0	17	64	40
2	88	♀	40	250	4.2	2.0	2.65	2.75	78.3	161.0	120.9	31	64	48	
3	83	♀	59	250	5.3	1.45	1.95	2.75	99.4	179.7	127.0	40	77	51	
4	79	♀	46	250	4.5	1.3	2.65	2.0	97...	148.0	116.0	39	59	47	
5	79	♀	44	250	5.1	0.6	1.75	2.75	47.8	95.1	101.9	19	38	41	
6	79	♀	54	250	4.5	1.2	1.43	1.83	76.7	130.9	141.7	31	52	57	
7	75	♂	69	250	4.0	1.0	1.75	1.8	115.1	130.2	177.6	46	57	71	
8	65	♀	82	375	4.9	1.15	1.25	1.15	109.4	177.1	229.0	29	47	88	
9	70	♀	81	250	3.5	0.83	1.05	1.25	151.1	204.3	163.9	60	82	66	
10	82	♀	65	250	6.3	1.13	1.95	1.75	102.8	156.5	131.9	41	63	53	
11	84	♀	61	250	7.4	0.3	1.3	1.25	87...	129.0	121.5	35	57	49	
Mean	77		61	261	5.0	1.04	1.77	1.91	91.6	151.9	144.3	34	59	55	
+S.E.M.			4	11	0.3	0.15	0.16	0.18	9...	9.0	13...	4	6	3	

were carried out on two consecutive days in 11 patients during maintenance therapy with three different preparations. The correlation coefficient (r) was +0.60 and the correlation is highly significant ($p < 0.001$). Digoxin clearance is on an average 73 ml/min/1.73 m² throughout the material and does not deviate significantly when measured separately during each therapy (S 76 W 74 L 69 ml/min/1.73 m²). Renal excretion was thus dependent on absorption and, in spite of decreased kidney function increased serum digoxin levels caused an increase of digoxin excretion into the urine.

A comparison was made of the combined means of the serum digoxin levels in stages I and II. The values obtained are shown in Table II. The concentrations in individual patients during maintenance therapy are based on 2-4 determinations. Differences S-W and S-L are statistically highly significant ($p < 0.001$) the difference W-L is not significant. Compared with preparation S the average mean obtained with W was 62% higher and with L 75% higher.

DISCUSSION

Our study shows that when one digoxin preparation is changed to another with better absorption during maintenance therapy the improved absorption will be greatly compensated by increased ex-

cretion of digoxin into the urine also in all these elderly patients. Although none of our patients were uraemic and their serum creatinine levels were normal the average creatinine clearance was low. When the steady state digoxin level in the serum with preparation W was on an average 70% and with L 84% higher than with preparation S the digoxin excretion into the urine was respectively 66 and 58% higher.

As far as we know only in the study of Falch et al. (1) has digoxin excretion into the urine been used in a comparison of bioavailability of digoxin. These investigators compared the new and old preparations of Lanoxin. In our study preparation W is the same new Lanoxin preparation as used by them. Falch et al. found that 46% of the daily digoxin dose was excreted into the urine with this preparation during 4 hours, while our material showed 59%. The patients of

Table II *Mean steady state serum digoxin concentrations in the total material*

	Preparation		
	S	W	L
Mean	0.98	1.59	1.77
No. of pats.	36	38	30
S.D.	0.38	0.51	0.75
S.E.M.	0.06	0.10	0.14

EFFECT OF NICOTINIC ACID ON MUSCLE LIPID CONCENTRATION IN MAN

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Abstract Muscle biopsies have been taken by needle biopsy from the femoral muscle of 11 subjects with hyperlipidemia before and during nicotinic acid treatment. No consistent changes were observed in the muscle triglyceride (TG) concentration during nicotinic acid treatment when all subjects were regarded as group. In subjects with high initial TG values in muscle (40.0-76.1 $\mu\text{mole/g}$, $n=5$) however decrease and in subjects with initial low values (8.9-14.3 $\mu\text{mole/g}$, $n=7$) an increase was observed during nicotinic acid treatment.

Nicotinic acid lowers the concentration of cholesterol and triglycerides (TG) in plasma (5, 6) and has found use as a drug in the treatment of hyperlipidemia (3, 7). Little information is however available on the effect of long-term administration of nicotinic acid on the lipid metabolism in tissues. Carlson et al have previously observed reduced levels of TG in myocardium, skeletal muscle and liver in rats after acute administration of nicotinic acid (4) and there is evidence that nicotinic acid administration to hyperlipidemic subjects may lower the TG concentration also in femoral muscle (8). A report is here given on the concentration of cholesterol and TG in skeletal muscle of man during treatment with nicotinic acid.

SUBJECTS

The subjects were obtained among participants in health survey study in Stockholm. Those with elevated levels of total plasma lipids were admitted to the clinic as outpatients for further investigation of their plasma lipid metabolism. Twelve of them, 7 women and 5 men, volunteered for muscle biopsy and were given nicotinic acid (Nicotin® Draco, Sweden), 1.5 g three times a day in order to lower the plasma lipid concentration. All subjects were in full-time work. No advice with regard to plasma lipid lowering regimes had been given before nicotinic acid treatment.

Table I gives some anthropometric data of the subjects and their types of hypertriglyceridemia (1) determined by paper electrophoresis and separation of the plasma lipoprotein fractions by ultracentrifugation before treatment. All had normal values with regard to concentration of glucose in blood, transaminases, alkaline phosphatases and uric acid in plasma.

METHODS

Sampling was done between 8 and 9 a.m. after overnight fast before and during nicotinic acid administration. The concentration of TG and cholesterol in plasma was determined by the AutoAnalyzer technique (11). Muscle tissue was taken by needle biopsy from the vastus lateralis of the femoral muscle according to Bergström (*). The muscle tissue was dissected at magnification of 40 under dissection microscope to remove visible adipose tissue and connective tissue. After freeze-drying, the muscle material was divided into 7 pieces, separately extracted for lipids, and the concentration of TG and cholesterol was determined as previously described in detail (10). A mean value for the concentration of cholesterol and TG in each muscle sample was then calculated from the separate determinations and used in the tables. All muscle values are given on dry weight basis.

Evaluated by analysis of variance the S.D. for the TG concentration in muscle sample was found to be 5 $\mu\text{mole/g}$, for cholesterol 0.25 mg/g.

RESULTS

As evident from Table II muscle biopsies were taken with a mean interval of 6, 1 and 16.3 weeks. The mean values for the plasma TG concentration decreased consistently during nicotinic acid treatment. The cholesterol concentration decreased from mean of 337 to 266 mg/100 ml after 11 weeks of treatment and remained at this level with continued nicotinic acid administration. No significant changes were observed in the mean concentration of TG or cholesterol in muscle tis-

Table I Anthropometric data and types of hyperlipidemia

	N	Age (y.)	Index ^a	Weight (kg)	Type ^b			
					N	II A	II B	IV
Women	7	52.6±3.5	113±0.10	70.4±5.5	1	5	1	
Men	5	49.4±4.5	107±0.05	74.4±1.9	1	1	1	2
Total	1	51.3±...7	111±0.06	72.1±3.6				

$$\frac{\text{weight}}{\text{height}^2} \times 100$$

According to Baumont et al. (1): N=normal lipoprotein pattern

sue at the different times of sampling when all subjects were regarded as one group ($n=1$).

The muscle TG concentration before nicotinic acid administration was 37.7 ± 9.6 $\mu\text{mole/g}$ in the women (range 74.8–14.3) and 26.7 ± 1.6 $\mu\text{mole/g}$ in the men (range 76.1±8.9). No correlation was found between the TG concentration in muscle and plasma ($r=0.22$, $p>0.05$).

Fig. 1 gives the individual percent change in the muscle TG concentration during nicotinic acid treatment. As evident from the Figure the change in the muscle TG concentration decreased in 5 and increased in 7 subjects. In the subjects with the marked initial increase of muscle TG concentration during nicotinic acid treatment the absolute values changed from 8.9 to 31.7 $\mu\text{mole/g}$ (–56%) and from 4 to 73 $\mu\text{mole/g}$ (+77%), respectively.

When further analysed the decrease in the muscle TG concentration was observed only in subjects with initially high values (range 76.1–40.5 $\mu\text{mole/g}$) and the increase only in subjects with low initial values (range 8.9–22.4 $\mu\text{mole/g}$). Calculated as absolute values the mean muscle TG concentration was reduced from a mean of 58 to 30.9 $\mu\text{mole/g}$ after 6 weeks of treatment in

subjects with initially high values while an increase was found from a mean of 15 to 30.9 $\mu\text{mole/g}$ after a similar period of treatment in subjects with initially low values (Table II). Treatment for more than 6 weeks caused no further change in the muscle TG concentration. The concentration of cholesterol in muscle remained unchanged during nicotinic acid treatment in the two groups arbitrarily formed on the basis of the muscle TG concentration (Table III).

DISCUSSION

Nicotinic acid administration lowered the plasma TG concentration in all subjects but had varying effects on the muscle TG concentration. With high initial values for the muscle TG concentration a decrease was observed, with low an increase. Fröberg (8) has previously reported a positive relationship between the decrease and the initial muscle TG concentration in male hyperlipidemic subjects after a period of 6 weeks of nicotinic acid administration.

The divergent effect of nicotinic acid treatment on the muscle TG concentration observed here suggests that the metabolism of muscle TG is not

Table II Sample interval and concentration of TG in plasma (TGpl) and in muscle (TGM) and of cholesterol in plasma (Cholpl) and in muscle (Cholm) during nicotinic acid treatment

	Sample no.			
	1	2	3	4
No. of subjects	1	11	10	4
Weeks	0	6.0±0.4	12.0±0.7	16.3±1.7
TGpl (mmole/l)	3.71±1.02	1.60±0.28	1.46±0.22	1.18±0.10
Cholpl (mg/100 ml)	337±9.8	256±15.8	205±15	277±9
TGM (μmole/g)	33.1±7.1	31.7±5.4	28.2±4.0	27.6±4.1
Cholm (mg/g)	1.96±0.10	2.24±0.17	1.89±0.17	1.80±0.13

Table III. Muscle lipids during nicotinic acid treatment in subjects with initially high and with initially low values for the muscle TG concentration

No. of subjects within parentheses. Abbreviations as in Table I

	Sample no.			
	1		3	4
TGm (μ mole/g)				
High	58.2 \pm 7.4 (5)	33.2 \pm 8.9 (4)	35.6 \pm 11.3 (3)	—
Low	15.2 \pm 1.7 (7)	30.9 \pm 7.3 (7)	25.5 \pm 3.5 (7)	27.6 \pm 4.1 (4)
Cholm (mg/g)				
High	2.03 \pm 0.15	2.32 \pm 0.44	1.90 \pm 0.34	—
Low	1.91 \pm 0.14	2.17 \pm 0.16	1.89 \pm 0.1	1.80 \pm 0.15

directly related to the metabolism of plasma TG. This is also in accordance with the lack of correlation between the concentration of TG in plasma and muscle previously observed in subjects with elevated plasma lipid levels (8). This is at variance with findings in men with normal levels of plasma TG 2.0–2.1 mmole/l, where a positive although weak correlation was found between the concentration of TG in plasma and muscle (9, 10). Interestingly in healthy subjects (10) the mean value for the muscle TG concentration was 29.1 \pm 1.3 μ mole/g dry weight ($n=47$) a value of the same order of magnitude as was approached in the present study during nicotinic acid treatment.

Studies on the metabolism of the muscle TG may reveal another facet of lipid metabolism which in clinical work is usually defined and evaluated on the basis of the pattern and metabolism of the plasma lipids. The different response observed in subjects with low and with high initial muscle TG concentration during nicotinic acid treatment does not argue against this concept. Further studies are however necessary to evaluate this aspect.

ACKNOWLEDGEMENTS

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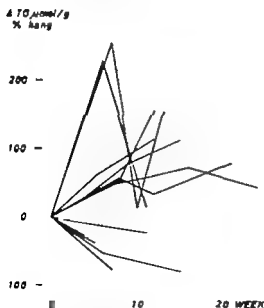


Fig. 1. Percent change in muscle TG concentration during nicotinic acid treatment.

DEMONSTRATION OF AN ENHANCED α -ADRENERGIC RESPONSE IN HUMAN ADIPOSE TISSUE WHEN INCUBATED IN PRESENCE OF Li^+ INSTEAD OF Na

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Abstract A study has been made of the effect of Li^+ on the adrenergic receptor response in human adipose tissue. Subcutaneous adipose tissue was preincubated and incubated in media in which Na⁺ had been replaced by Li^+ . The adrenergic response of the tissue was assayed by measuring the rate of lipolysis stimulated with noradrenaline in the presence or absence of phenolamine. The capacity of the hormone-sensitive lipase was measured by the use of dibutyryl cyclic AMP. The results showed that the α -adrenergic response was enhanced by the presence of 60 mM Li^+ while the β -adrenergic response was affected only in a higher concentration of Li^+ (120 mM). The response of the lipase enzyme to dibutyryl cyclic AMP remained unaffected by Li^+ however. The effect of Li^+ was inhibited by 0.5 mM La^{+++} in the medium, indicating that Li^+ had increased the entry of Ca^{++} into the cells. These results suggest that the α -adrenergic response of human adipose tissue depends on the intracellular concentration of Ca^{++} for its inhibitory action on the adenylyl cyclase enzyme.

It has previously been demonstrated that the lipolytic response to noradrenaline *in vitro* is markedly diminished in adipose tissue from hypothyroid subjects. The decrease was shown to be due to an enhancement of the α -adrenergic receptor response of the tissue (19) which inhibited the formation of cyclic AMP (9). A similar increase in α -adrenergic response was also demonstrated in aortic smooth muscle from hypothyroid rabbits (18). How the lack of thyroid hormones renders the tissue more responsive to α -adrenergic stimulation is however not known at present. It has been suggested that it might be the result of a change in the intracellular ion content (17). This hypothesis is supported by the demonstration that more Ca^{++} was liberated by α -adrenergic stimulation from intracellular pools in smooth muscle from hypothyroid than control strips (18).

The present study was undertaken in order to examine whether the α -adrenergic response in normal human adipose tissue could be enhanced by an increase in the intracellular concentration of calcium.

METHODS

Subcutaneous adipose tissue was obtained from subjects undergoing cholecystectomy. None of the patients showed evidence of diabetes mellitus or other metabolic disease. The biopsies were removed after induction of general anaesthesia by Nembutal® (pentobarbital sodium) nitrous oxide and Halothane® (bromochlorotrifluoroethane). The tissue was transported in 0.9% NaCl from the operating room and incubated as previously described (8).

The tissue was preincubated for one hour in Krebs-Henseleit bicarbonate buffer (KHB) (pH 7.4) containing 1% bovine albumin. Separate 50-100 mg pieces were then transferred to plastic vials containing 1.5 ml incubation medium made of KHB with 3% albumin, 1 mg/ml glucose (pH 7.4). The incubation were carried out at 37°C for two hours using air as the gas phase. The release of glycerol was used as an index of lipolysis and analyzed according to Chernick (5).

In experiments in which Li^+ was used, NaCl was replaced by equal amounts of LiCl. In order to block the entry of extracellular Ca^{++} into the cells the rare earth metal lanthanum (La^{+++}) was added to the preincubation and incubation media. In this series of experiments the medium of van Breemen and McNaughton (4) was used instead of KHB since the presence of phosphate and bicarbonate in the KHB would have formed insoluble salts with La^{+++} . The medium had the following composition: 160 mM NaCl or 160 mM LiCl, 4.6 mM KCl, 1.5 mM CaCl_2 , 1 mM MgCl_2 and 5 mM Tris-HCl (pH 7.4). The same concentration of albumin and glucose were used in the preincubation and incubation media as in the experiments with KHB.

The following pharmacological agents were used in the incubations: L-noradrenaline bitartrate (Astra, Ss

Table 1 Effect of different concentrations of Li^+ in the preincubation media on basal lipolysis (mean \pm S.E., $N=6$)

	Concentration in medium				P
Li (mM)	0	30	60	120	
Glycerol ($\mu\text{moles/g ww}/2\text{h}$)	0.342 ± 0.07^a	0.314 ± 0.091	0.252 ± 0.095	0.193 ± 0.043	N.S.

Calculated from two-way analysis of variance (11).

den) phentolamine (Regitin, Ciba, Switzerland) and $N^6,2'$ -dibutyryl cyclic 3',5'-adenosine monophosphate (Boehringer/Mannheim, West Germany).

Statistical calculations were performed on a PDP 8L computer using the FOCAL statistical routines of McKerrow (13) or the two-way analysis of variance programme by Hall (11).

RESULTS

Replacing Na by Li in the preincubation and incubation medium did not significantly reduce the basal glycerol release from the tissue (Table I).

The effect of different concentrations of Li^+ on the net lipolytic response to 2×10^{-6} M noradrenaline, 1×10^{-6} M noradrenaline plus $5 \mu\text{g/l}$

ml phentolamine or 10^{-6} M dibutyryl cyclic AMP is shown in Fig. 1. Increasing the concentration of Li significantly and proportionately reduced the noradrenaline response, while the responses to noradrenaline plus phentolamine or dibutyryl cyclic AMP were not significantly modified.

The effect of 60 and 120 mM Li on the dose response to noradrenaline in the presence and absence of phentolamine is shown in Figs. 2 and 3. At a Li concentration of 60 mM the noradrenaline response was markedly reduced while that to noradrenaline plus $5 \mu\text{g/ml}$ phentolamine remained unaffected (Fig. 2). At a higher concentration of Li (120 mM) however the response both to noradrenaline and to noradrenaline plus $5 \mu\text{g/ml}$ phentolamine was reduced as shown in Fig. 3. Only the highest concentration of noradrenaline used (14×10^{-6} M) gave a significant stimulatory effect in the presence of phentolamine.

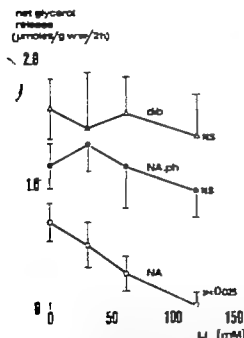


Fig. 1 Effect of different concentrations of Li^+ in the preincubation and incubation media on the net lipolytic response to 10^{-6} M dibutyryl cyclic AMP (dib) (Δ - Δ), 2×10^{-6} M noradrenaline (NA) (\circ - \circ) and 2×10^{-6} M noradrenaline plus $5 \mu\text{g/ml}$ phentolamine (NA.ph) (\bullet - \bullet) (mean \pm S.E., $N=6$). The p -values were calculated from a one-way analysis of variance (11).

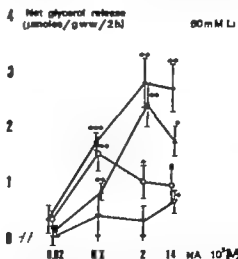


Fig. 2 Effect of 60 mM Li^+ on the net lipolytic response to different concentrations of noradrenaline (NA) in presence (\circ - \circ , \bullet - \bullet) and absence (Δ - Δ , Δ - Δ) of $5 \mu\text{g/ml}$ phentolamine (mean \pm S.E., $N=5$). Filled symbols denote the results obtained in presence of Li while the unfilled symbols show the results in the normal KHB buffer. $p < 0.05$, $p < 0.01$, $p < 0.001$.

4 Net glycerol release
($\mu\text{moles/g ww}/2\text{ h}$)

120 mM Li

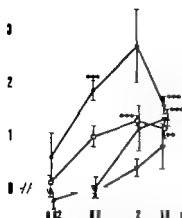


Fig. 3 Effect of 120 mM Li^+ on the net lipolytic response to 1-noradrenaline (NA) in presence and absence of 5 $\mu\text{g/ml}$ phentolamine (mean \pm S.E. $N=5$). Symbols and p -values as in Fig. 1.

The presence of 0.5 mM La^{+++} in preincubation and incubation media modified the effect of Li^+ on the adrenergic receptor response as shown in Fig. 4. When Na was replaced by Li^+ in the medium the response to $\times 10^{-8}$ M noradrenaline was markedly reduced while that to 2×10^{-8} M noradrenaline plus 5 $\mu\text{g/ml}$ phentolamine or dibutyryl cyclic AMP remained unaffected. The inhibitory effect of Li^+ on the noradrenaline response was inhibited by the presence of 0.5 mM

La^{+++} . The response to $\times 10^{-8}$ M noradrenaline plus 5 $\mu\text{g/ml}$ phentolamine or 10^{-6} M dibutyryl cyclic AMP was not affected by the presence of La^{+++} .

DISCUSSION

The present investigation was undertaken in order to examine whether a change of the intracellular ion content would affect the α -adrenergic response of human adipose tissue. The results show that the α -adrenergic response of the tissue could be selectively enhanced by substituting 60 mM Na for Li^+ in the medium. The effect of Li^+ was most probably due to an increased accumulation of Ca^{++} in the cells.

It has previously been demonstrated that calcium ions accumulate intracellularly if tissue specimens are incubated in sodium-deficient media. This has been shown for squid axons (1) smooth muscle (3) cardiac muscle (15) rat adipose tissue and brain tissue (6, 20). From these studies it seems well established that the active transport of calcium out of these cells depends on a gradient of sodium over the cell membrane while the flow of Ca^{++} into the cells is passive. Li^+ , choline and sucrose do not participate in this sodium-calcium exchange and hence calcium will accumulate when sodium is replaced by any of these substances. So far no evidence of such a sodium-calcium exchange mechanism has been demonstrated in hu-

NaCl-Tris

Net glycerol release
($\mu\text{moles/g ww}/2\text{ h}$)

3

- La^{+++}

+ La^{+++}

2

1

0

P

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

NA

NA+ph

db

LiCl-Tris

Fig. 4 Inhibitory action of 0.5 mM La^{+++} on the effect of Li^+ -containing media on the net lipolytic response to $\times 10^{-8}$ M noradrenaline (NA), 2×10^{-8} M noradrenaline plus 5 $\mu\text{g/ml}$ phentolamine (NA+ph) and 10^{-6} M dibutyryl cyclic AMP (db) (mean \pm S.E. $N=6$).

man adipose tissue. However, in view of the many tissues which have been shown to possess such a sodium-calcium exchange mechanism a sodium-deficient medium was tried as an instrument to increase the intracellular concentration of calcium in the present series of experiments. Although no direct measurements of the Ca^{++} uptake were made, the finding that La^{+++} could antagonize the effect of Li^+ favours the presence of such a sodium-calcium exchange mechanism in human adipose tissue. This conclusion rests on the observations that La^{+++} blocks the entry of Ca^{++} into cells by adhering to a cellular surface component which normally binds Ca^{++} (4). The sodium-potassium exchange, on the other hand, does not seem to be blocked by La^{+++} (1).

The replacement of Na^+ by Li^+ in the present study diminished the noradrenaline-stimulated lipolysis. The degree of inhibition depended on the concentration of Li^+ (Fig. 1). The lipolytic effects of noradrenaline plus phentolamine or dibutyryl cyclic AMP were however not significantly changed unless the concentration of Li^+ was high (120 mM) (Figs. 1 and 3). The effect of 60 mM Li^+ on the noradrenaline response and the absence of an effect on the response to noradrenaline plus phentolamine indicate that the α -adrenergic response was preferentially influenced at this concentration of Li^+ . At a higher concentration of Li^+ (J mM), however, both the responses to noradrenaline and noradrenaline plus phentolamine were affected, showing that the α - as well as the β -adrenergic responses were inhibited.

The present finding that the lipolytic effect of dibutyryl cyclic AMP was unaffected by Li^+ demonstrates that the effect of Li^+ does not set in at the level of lipase activation but at an earlier step in the lipolytic mechanism. Such a site could probably be the adenylyl cyclase, since our previous studies have shown that the α -receptor acts by inhibiting the formation of cyclic AMP (10). The effect of Li^+ could be the result of either an increased intracellular accumulation of Ca^{++} due to the sodium-deficient media, to an inhibitory effect of Li^+ per se or to a decreased intracellular concentration of K^+ . Both Li^+ and Ca^{++} are known to inhibit adenylyl cyclase while K^+ stimulates the enzyme system (2, 7). It was of importance to differentiate between these possibilities. In order to study whether the effect of Li^+ was due to the entry of Ca^{++} into the cells, La^{+++} was used as

an inhibitor of Ca^{++} influx. The concentration of La^{+++} (0.5 mM) used completely blocked the influx of Ca^{++} into K^+ -depolarized smooth muscle as previously shown (18). In the present experiments the effect of Li^+ on the α -adrenergic response was counteracted by the presence of La^{+++} in the medium (Fig. 4). This indicates that the effect of Li^+ on the α -adrenergic response can be attributed to the entry of calcium into the cells and not of Li^+ per se. Nor could the effect of Li^+ be due to a decrease in K^+ , since the β -adrenergic response elicited by noradrenaline in the presence of phentolamine was unchanged while the α -response was enhanced in the presence of 60 mM Li^+ .

In human adipose tissue α -adrenergic stimulation inhibits the formation of c AMP and lipolysis, while β -adrenergic agonists stimulate the synthesis of c AMP and lipolysis (8-10). The response to noradrenaline, a combined α - and β -adrenergic agonist, will therefore depend on the relative responsiveness of the two types of receptors in the adipocytes. The α -adrenergic receptor response can be modulated by the level of thyroid hormone. Thus specimens of adipose tissue from hypothyroid subjects showed a marked increase in the α -adrenergic responsiveness which completely inhibited the normal lipolytic effect to noradrenaline (19). The β -adrenergic response as measured by stimulation with isopropyl noradrenaline or noradrenaline in the presence of phentolamine, however, remained intact, as did also the hormone sensitive lipase enzyme (19).

The mechanisms behind the increase in α -adrenergic response in adipose tissue from hypothyroid subjects is not known at present. Earlier studies, however, have demonstrated that the hypothyroid state in human subjects is accompanied by an increased intracellular concentration of sodium (1). The underlying mechanism could possibly be a decreased Na^+/K^+ ATPase activity of the cells as demonstrated in hypothyroid rats (1). Such an enhanced intracellular concentration of Na^+ might in turn increase the concentration of Ca^{++} in the cells (17). Since the Ca^{++} was higher in smooth muscles from hypothyroid rabbits than controls upon α -adrenergic stimulation (18) it was postulated that the enhanced α -adrenergic response observed in human adipose tissue from hypothyroid subjects could also be due to such a derangement of the intracellular Ca^{++} ion content.

(17) The present finding that Li could increase the α -adrenergic response and that the effect of Li⁺ was blocked by the presence of La⁺⁺⁺ favours the hypothesis that calcium is involved in the α -adrenergic response in human adipose tissue. Furthermore since it was shown previously that the difference in noradrenaline response between normal adipose tissue and that from hypothyroid subjects was due to a quantitative rather than qualitative change in adrenergic response (16) it might be postulated that the observed enhancement of the α -response in tissues from hypothyroid subjects could also be due to an increased concentration of Ca⁺⁺ upon α -adrenergic stimulation. In this context it is of interest to note that ouabain, which blocks the Na⁺ K⁺ ATPase inhibited the lipolytic response to noradrenaline in the human forearm preparation in healthy subjects (14).

The present study has thus shown that the α -adrenergic response in human adipose tissue can be enhanced in vitro by the presence of Li in the incubation media. The change induced by Li was most probably the result of an increased accumulation of calcium intracellularly since it could be antagonized by La⁺⁺⁺. Whether α -stimulation is expressed through an increase in ionized calcium intracellularly or whether it increases the affinity of Ca⁺⁺ for the adenylyl cyclase however cannot be determined from the present study.

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PROTEINURIA IN NON RENAL INFECTIOUS DISEASES

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Abstract. The urinary excretion of total protein, orosomucoid, albumin, transferrin, and IgG, has been determined in 19 children and 23 adult with various non-renal infectious diseases. Furthermore urine osmolality was estimated in 39 of these patients (16 children 23 adults). An often considerably increased urinary protein excretion ("febrile proteinuria") was found in 74% of the children with maximal output of 2283 mg/24 h and in 83% of the adults, maximal output 3350 mg/24 h. In all cases an increased excretion of orosomucoid was found, but in many cases the excretion of the other proteins determined was increased as well, which excludes prerenal mechanisms as the only factor responsible for the increased protein excretion. The hypothesis is put forward that infections result in an immunological injury of the glomerular and/or tubular basement membrane, which might be of pathogenetic importance in the development of renal diseases.

Different factors among them fever influence the normal urinary protein excretion. The term "febrile proteinuria" is used extensively but only a few investigations concerning this subject have been published. Weitz (24) examined the urinary protein excretion in 40 patients undergoing fever therapy. In 78% of the cases a substantial increase in the protein excretion was found. Ehrström (5) demonstrated proteinuria in 36% of 300 patients with different infectious diseases. The highest output of protein was found in patients with pneumococcus and influenza infections. Marks et al (12) investigated urine protein in 198 children with febrile illnesses. Proteinuria was found in 5.6%.

Thus earlier investigations have in most cases determined the urinary excretion of total protein, and the results of these determinations have been somewhat divergent. Specific immunochemical methods for quantitation of different plasma proteins are now available. Therefore we examined the urinary excretion of total protein and 5 plasma

proteins in 42 patients suffering from various non-renal infectious diseases.

MATERIAL

The patients were admitted to an emergency section of the University Clinic for Infectious Diseases, Copenhagen. For practical reasons only one patient per day the first with rectal temperature $\geq 38^{\circ}\text{C}$ on admission, was chosen. To enter the study the patients so selected should fulfil the following criteria. 1) No kidney diseases in the family. 2) No previous or present kidney disease or generalized disease with known probable kidney affection. 3) Normal urine sediment by microscopy and no growth of bacteria in the urine.

The material comprised 42 patients, 11 females and 31 males aged 2 3/4-76 years, 19 < 15 years. Details of the patients are given in Table I.

The control material comprised 12 adult persons from the laboratory and medical staff, 7 females and 5 males aged 25-69 years. Nobody had previous kidney diseases or signs of present infectious diseases. Their Hb concentration, ESR, serum paper electrophoresis and serum creatinine were normal. The urinary sediments were normal and there was no bacterial growth in the urines.

METHODS

The patients urine was quantitatively collected from 11 p.m. after admission and during the following 4 hours. In one patient (no. 24) 17 consecutive 24 h portions of urine were collected.

During collection the urines were stored at 4°C and sodium azide was added as preservative to give final concentration of 0.1% (w/v). During collection of urine the patients were confined to bed. In the middle of the collection period blood sample was drawn and from this serum was used. Mean temperature signifies the temperature at the start of the collection period plus the temperature at the termination of collection period divided by 2.

Immediately after admission material from the infected organ was obtained for microscopy and cultivation and appropriate antibiotic therapy was instituted.

Table 1 *Clinical and laboratory data of 47 patients with various infectious diseases*

NI=not identified

Pat. no	Sex	Age (y)	Diagnosis	Infecting micro-organism	Mean temp. (°C)	Cl _{cr} (ml/min)	Albustix® reaction	Urine protein (mg/24 h)	Agarose-gel pattern ^a
1	♂	2 3/4	Bact. meningitis	Neiss. mening.	37.0	15	—	117	B
2	♂	4	Lymph. meningitis	NI	38.5	37	Trace	264	C
3	♂	4	Lymph. meningitis	Mumps virus	39.5	0.7 ^m	Trace	143	B
4	♂	5	Bact. meningitis	Haem. influen.	36.6	22	Trace	1 232	E
5	♂	6	Tonsillitis	NI	38.8	0.7 ^m	—	60	A
6	♂	6	Bact. meningitis	NI	38.	2.03 ^a	—	383	C
7	♀	6	Pharyngitis	NI	39.0	66	Trace	91	A
8	♂	8	Tonsillitis	Haem. haemolyt.	37.1	66	Trace	56.	E
9	♀	8	Pharyngitis	Haem. haemolyt.	37.9	23	—	63	A
10	♂	9	Lymph. meningitis	Mumps virus	37.8	31	Trace	97	A
11	♂	9	Inf. mononucleos.		38.8	91	Trace	230	A
12	♂	10	Lymph. meningitis	Mumps virus	39.6	49	—	151	B
13	♂	10	Pneumonia	NI	38.3	89	Trace	138	B
14	♀	11	Malaria	Plasmod. vivax	38.7	57	Trace	47	A
15	♂	11	Lymph. meningitis	Mumps virus	38.5	66	—	167	A
16	♂	13	Pneumonia	NI	39.6	44	Trace	490	D
17	♀	13	Pneumonia	Pneumococcus	38.2	83	+	1 910	E
18	♂	15	Febrile causa ign.	NI	38.8	158	—	401	B
19	♂	15	Pneumonia	Pneumococcus	37.8	105	+	2 283	C
20	♂	16	Sepsis	NI	39.0	103	Trace	367	B
21	♂	16	Lymph. meningitis	NI	38.5	156	+	366	B
22	♂	17	Bact. meningitis	Neiss. mening.	37.8	150	Trace	784	C
23	♂	2.	Tonsillitis	NI	39.4	118	+	708	C
24	♂	22	Bact. meningitis	Neiss. mening.	39.7	165	+	2 687	C
25	♀	25	Lymph. meningitis	NI	38.1	72	Trace	426	D
26	♂	26	Malaria	Plasmod. vivax	38.9	61	Trace	390	C
27	♂	26	Mumps	Mumps virus	37.5	108	Trace	1 360	D
28	♂	32	Acute gastroent.	Salm. sero-typ. kangi	37.7	33	Trace	30	A
}	♂	33	Postvacc. encephalit.		38.5	52	—	1.1	A
	♂	34	Impetigo	Haem. strept.	37.7	135	+	750	C
	♂	42	Mumps	Mumps virus	40.6	97	+	1 690	E
	♂	42	Bact. meningitis	Pneumococcus	38.5	105	++	3 350	E
	♀	43	Acute gastroent.	Salm. typ. marium	38.7	66	+	486	C
34	♂	44	Acute gastroent.	NI	38.6	84	Trace	1 212	C
35	♂	49	Bact. meningitis	Pneumococcus	39.3	90	Trace	1 333	D
36	♀	50	Febrile causa ign.	NI	37.6	84	—	113	A
37	♀	56	Pharyngitis	Haem. influen.	38.1	94	Trace	95	A
38	♂	60	Bact. meningitis	Pneumococcus	38.5	66	+	2 180	E
39	♀	6.	Bact. meningitis	Pneumococcus	37.7	84	Trace	694	F
40	♂	67	Malaria	Plasmod. malariae	38.9	2.74 ^a	++	530	D
41	♀	73	Pneumonia	NI	38.6	101	+	1 010	E
42	♀	76	Bact. meningitis	NI	38.7	49	Trace	304	F

Serum creatinine (mg/100 ml).

A=normal B=broad α_2 and fast α_2 , C=tubular D=glomerular E=glomerulo-tubular F=renalisable

LABORATORY METHODS

The 24 h portions of urine were tested by means of Albustix® and aliquots were withdrawn for the following examinations. 1) Protein. A biuret method was used, slightly modified after Sørensen et al. (17) 2) Microscopy cultivation and determination of creatinine

The residue of the 24 h portions was concentrated at 4°C by means of low pressure ultrafiltration using Viking dialysis tubes 23/37 inches (6). In the con-

centrated urines and in the serum samples the following proteins were quantitated by means of immunoelectrophoresis in antibody-containing agarose gel: orosomucoid, albumin, transferrin, IgG and immunoglobulin (11, 22, 23). The antibodies to the different proteins were obtained from Dakopatts A/S Copenhagen.

In order to evaluate the type of proteinuria, agarose-gel electrophoresis was performed in 0.075 M cross buffer of pH 8.4 containing 1% (w/v) agarose. The electrophoresis was run for 45–60 min at 20 V/cm.

Table II *Proteinuria serum concentration and urinary excretion (mean and observed range) of 5 plasma proteins in 12 controls*

	Total protein	Muramidase	Orosomucoid	Albumin	Transferrin	IgG
Serum (g/l)	—	— ^a 1.3–3.0	0.78 0.67–1.00	38.2 31.7–45.	— ^b 1.80–3.06	1.00 7.3–19.8
Urine (mg/24 h)	—	51 ^b 43–123	0.42 0.1–0.82	7.7 3.9–19.0	0.18 0.06–0.36	2.8 1.0–4.3
	mg/l.	µg/24 h				

RESULTS

Controls

The urinary protein excretion, the serum concentration and the urinary excretion of muramidase, orosomucoid, albumin, transferrin and IgG in the 12 control subjects are shown in Table II. The protein excretion was 43–123 mg/24 h. The mean urinary excretion of orosomucoid, albumin, transferrin and IgG was 0.47, 7.7, 0.18 and 2.8 mg/24 h, respectively. Urine muramidase was on an average 51 µg/24 h.

Patients

Albustix reaction. In 9 cases the Albustix reaction was negative, in 1 it showed a trace, in 10 cases + and in 1 case ++. In the 9 cases with negative Albustix reaction the urinary protein excretion varied from 52 to 401 mg/24 h. In the 1 case with trace reaction from 30 to 1369 and in the 10 cases with +reaction from 366 to 2687. In the 2 patients with ++reaction, the proteinuria was 530 and 3350 mg/24 h, respectively.

Urinary protein excretion. In the 19 patients <15 years the urine protein was 63–283 mg/24 h. In 14 cases it was above 123 mg/24 h (=highest value in the controls). In the 23 patients >15 years urine protein was 30–3350 mg/24 h, and in 19 cases it was increased above normal. In 17 patients with a mean temperature <38.5°C the mean urinary excretion of protein was 649 mg/24 h. In the remaining 25 patients with a mean temperature >38.5°C the figure was 757 mg/24 h.

In 11 of the 19 patients <15 years the infection could with certainty be characterized as bacterial in 7 and viral in 6. In the 23 patients >15 years it was possible to characterize the infection as caused by bacteria in 12 and by virus in 5. The

7 children with bacterial infection had an average proteinuria of 936 mg/24 h (63–2283) and the 6 with virus infection 175 mg/24 h (97–264). In the 1 adult patient with bacterial infection the proteinuria was on an average 1088 mg/24 h (30–3350) and in the 5 adults with viral infection 793 mg/24 h (11–1690). Table III shows the mean and range of proteinuria in relation to the infecting microorganism.

In patient 4, a 22-year-old man with meningococcal meningitis, the daily urinary excretion of total protein, orosomucoid, albumin, transferrin and IgG was followed for 17 days. The results are shown in Fig. 1.

Table III *Proteinuria versus infecting microorganism*

C=children, A=adults

Microorganism	No. of pts.	Proteinuria (mg/24 h)	
		Mean	Range
Pneumococcus	C 1	097	1910–283
	A 4	2139	694–3350
Meningococcus	C 1	117	—
	A 1	1736	784–647
Haemophilus influenzae	C 1	1232	—
	A 1	95	—
Haemophilus haemolyticus	C 1	313	63–66
	A —	—	—
β-haemolytic streptococcus	C —	—	—
	A 1	790	—
Other bacteria	C 1	383	—
	A 4	297	30–486
Mumps virus	C 4	140	97–167
	A 2	1525	1360–1690
Other virus	C 1	247	230–264
	A 3	304	121–426
Malaria	C 1	47	—
	A 1	460	390–530

Table IV Serum concentration and urinary excretion of 4 plasma proteins in 42 patients with infectious diseases

D=decreased N=normal, I=increased C=children A=adult
 Figures within parentheses = % of total

		No. of pts											
		Orosomucoid			Albumin			Transferrin			IgG		
		D	N	I	D	N	I	D	N	I	D	N	I
Serum concentration (g/l)	C	1 (6)	1 (6)	17 (88)	4 (21)	12 (63)	3 (16)	7 (17)	1 (63)	6 (0)	4 (71)	15 (79)	11 (0)
	A	1 (4)	1 (9)	20 (87)	4 (18)	18 (78)	1 (4)	13 (56)	10 (44)	0 (0)	3 (13)	20 (87)	0 (0)
Urine excretion (mg/24 h)	C	0 (0)	0 (0)	19 (100)	0 (0)	9 (47)	10 (53)	0 (0)	4 (71)	15 (79)	0 (0)	4 (21)	15 (79)
	A	0 (0)	0 (0)	23 (100)	1 (4)	4 (17)	18 (79)	0 (0)	4 (17)	19 (83)	1 (4)	2 (9)	26 (87)

Orosomucoid In the children the urinary excretion was 4-855 and in the adults 5-472 mg/24 h.

Albumin In the patients ≤ 15 years the urinary excretion varied from 9 to 410 mg/24 h and in the patients > 15 years from 2 to 1054 mg/24 h.

Transferrin In the two groups the urinary excretion was 0-10-32 and 0-10-74 mg/24 h respectively.

IgG In the group ≤ 15 years the urinary excretion was 1-2-94 and in the group > 15 years 0-8-1 mg/24 h.

The results of orosomucoid, albumin, transferrin and IgG determinations are summarized in Table IV.

Antimuramidase Serum and urine muramidase was determined in 39 (16 children, 23 adults) of the 42 patients. An increased urinary muramidase excretion ($> 100 \mu\text{g}/24 \text{ h}$) was found in 9 cases, 121-3900 $\mu\text{g}/24 \text{ h}$. Three of these 9 cases were ≤ 15 years. In 7 of the 9 cases the infecting micro-organism was identified: pneumococcus 4 cases, haemophilus influenzae 1, mumps virus 1 and combined infection with plasmodium malariae and plasmodium falciparum 1 case (Table V).

Urine concentration mg/1000 ml
 log to % of normal mean

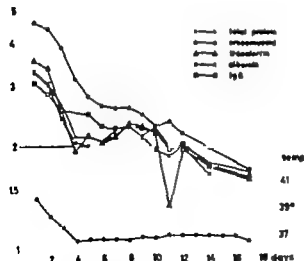


Fig. 1 Urinary excretion of total protein and 4 plasma proteins in a patient with meningococcal meningitis.

Agarose-gel electrophoresis of the urines

By means of agarose-gel electrophoresis of the urines it was possible to differentiate between five patterns of proteinuria: A=normal ($n=11$), B=normal except for a broad α_2 -band (presumably orosomucoid) and a distinct fast α_2 -band ($n=7$), C=tubular pattern ($n=10$), D=glomerular pattern ($n=5$), E=mixed glomerulo-tubular ($n=7$) and F=unclassifiable ($n=2$). All the urines with patterns C, D and E except one also showed pattern B. Fig. 2 shows the different patterns.

Thus an abnormal pattern was found in 73% of the urines examined. Among the children 12 urines (63%) belonged to the first two patterns, 7 (37%) to the last three, while among the adults 6 (26%) had the first two patterns, and 17 (74%) the last three (Table I).

Table V Data of 9 patients with muramidasuria

NI=not identified

Pat. no.	Diagnosis	Infecting microorganism	Creatinine clearance (ml/min)	Leukocytes / μ l	Proteinuria (mg/24 h)	Muramidase	
						Serum (mg/l)	Urine (μ g/24 h)
4	Bact. mening.	Haem. influen.	22		1.3	—0	160
17	Pneumonia	Pneumococcus	83	11 400	1 910	3.7	2 010
19	Pneumonia	Pneumococcus	105	25 600	283	4.5	2 700
31	Mumps	Mumps virus	97	11 200	1 690	4.7	1 130
32	Bact. mening.	Pneumococcus	105	13 600	3 330	3.9	3 900
34	Acute gastroent.	NI	84	7 800	1.1	6.3	496
35	Bact. mening.	Pneumococcus	90	15 900	1 333	—1	305
40	Malaria	Plasma malariae	—74*	10 800	530	6.6	650
		Plasma falc.					
41	Pneumonia	NI	101	—	1 010	3.3	121

Serum creatinine (mg/100 ml)

DISCUSSION

In the present study an adult control material was used both for children and adults. However as can be seen in Table I, a lowering of the upper limit of protein excretion in the group ≤ 15 years due to the supposedly lower normal protein excretion in children compared with adults (8) will cause only minimal elevation of the percentage of "febrile proteinuria" in children, as the urinary protein excretion in this group was either normal or markedly elevated. The results obtained in the present control material are in accordance with those of earlier investigators (2, 3, 16).

In the patients proteinuria (>123 mg/24 h) was

found in 74% of the children and in 83% of the adults. Ehrström (5) found "febrile proteinuria" in only 36%. But it is difficult to compare his results with those from the present study since the method of Esbach used by Ehrström gives variable results and probably excludes the more moderate "febrile proteinurias". Marks et al (12) found "febrile proteinuria" in only 5.6% of febrile children. This low percentage can in part be explained by their mode of selection which was based on an Albustix reaction >30 mg/100 ml corresponding to ++ or more in the present study. But Albustix reacts poorly with orosomucoid and immunoglobulins. If this method had been used in the present study 30 patients would have been excluded resulting in a lowering of the percentage of patients with "febrile proteinuria" to 29. Marks et al (12) found a positive Albustix reaction (as defined above) in 37 of 198 patients but only in 11 of these 37 cases was the sulphosalicylic acid test positive. Therefore they stressed the importance of a high incidence of false positive results obtained by means of Albustix but, as mentioned above they did not take into account the false negative reactions which are equally common.

Agarose-gel electrophoresis of the urines of 9 patients with normal urinary protein excretion revealed a normal pattern in 8 whereas in 1 patient the pattern was that of a broad α_1 -band and an accentuated α_2 -band. In contrast 3 patients with an increased protein excretion showed a normal pattern of agarose-gel electrophoresis. Thus in most cases a quantitatively abnormal protein ex

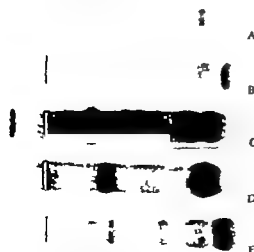


Fig. 2. Different patterns of proteinuria obtained by means of agarose-gel electrophoresis.

cretion corresponded to a qualitatively abnormal one.

In the children the proteinuria was greater in patients with bacterial than in those with viral infections. This difference was not found in the adults. Among the bacterial infections those caused by pneumococci showed the greatest proteinuria. These findings are identical with those of Ehrström (5). Also mumps infection in adults was accompanied by a substantial rise in protein excretion.

It has earlier been claimed that "febrile proteinuria" is almost exclusively prerenal (9). In the present study an augmented excretion of orosomucoid was found in 100% of the patients but in most cases the urinary excretion of albumin, transferrin and IgG was also increased. The augmented excretion of orosomucoid could be explained by a prerenal mechanism as the serum concentration of this protein was increased in most cases.

The antiserum used to quantitate IgG was specifically directed to the H chain of IgG. Consequently this antiserum reacts with free H chains or fragments of H chains. Vaughan et al. (20) found that about 15% of γ -globulins in urine from 4 persons represented the Fc fragments of IgG. In the present study it is most probable that part of this fraction designated IgG represents Fc fragments of IgG which, owing to their lower molecular weight, could cause a prerenal proteinuria. However, the increased excretion of albumin and transferrin indicates a renal (glomerular or tubular) lesion.

How far the febrile illness results in a glomerular and/or tubular injury was evaluated by measuring the urinary excretion of muramidase and by means of agarose-gel electrophoresis performed on the urines.

At normal serum concentration an increased urinary excretion of muramidase is a sign of lesion of the proximal tubules (1, 15). In the present study muramiduria was found in 9 of 39 cases investigated. As the serum concentration of muramidase in no case exceeded the renal threshold for muramidase, the finding indicates proximal tubular damage. The febrile illness in the 9 patients was caused by bacteria as well as by virus and malaria parasites. In 4 cases the infection was caused by pneumococci and in these cases the greatest muramiduria was found.

Of the 9 patients with muramiduria agarose-gel electrophoresis showed a tubular or glomerulo-tubular pattern in 7. In the remaining 2 the pattern was glomerular. Of the 15 patients with tubular or glomerulo-tubular pattern only 7 had muramiduria. Thus the correlation between these investigations was poor. Which if any of the above mentioned methods permits the diagnosis of tubular damage cannot be evaluated from the present study.

The renal lesion caused by an infectious disease is probably transitory. In only 1 patient was the daily urinary excretion of different proteins determined for a lengthy period—17 days. The result of this investigation showed that the urinary excretion of different proteins was not normalized until the 10th–14th day, whereas the temperature was normal on day 4.

Based on the above mentioned findings it can be concluded that an infectious disease in many cases results in a lesion of the glomeruli and/or tubules. The mechanism for this injury is unknown. A possible pathogenic factor is the elevated body temperature accompanying the infectious disease. Thus Wetly (24) found an increased urine protein excretion in 78% of 40 patients in whom fever was induced by means of the wetting hypertherm. Ehrström (5) showed that the height and duration of the fever did not influence the magnitude of proteinuria in patients with infectious diseases, whereas Marks et al. (12) found proteinuria only in patients with a body temperature above 38.4°C. The results in the present study did not favour the view of body temperature as the only factor of importance for the augmented protein excretion in the urine. Thus no difference in average proteinuria was found in 17 patients with a mean temperature <38.5°C nor in 25 patients with a mean temperature $\geq 38.5^\circ\text{C}$. Furthermore, in the patient in whom proteinuria was followed for 17 days the protein excretion did not become normalized until several days after the body temperature (Fig. 1).

Since fever alone cannot explain the "febrile proteinuria", one might propose renal damage by bacteria or virus, possibly by means of an immunological mechanism. One may advance the hypothesis that all infections result in the formation of antigen-antibody complexes in which the antigen is the infecting microorganism or parts of it and the antibody consists of immunoglobulins.

produced by the host against the antigens. These complexes are deposited on the epithelial side of the basement membrane and result in lesion of the membrane. How far this lesion results in a transient (febrile proteinuria) or more permanent (glomerulonephritis) abnormality depends, among other things, on the phagocytic function of the mesangium. If this function is undisturbed the immune complexes will be taken up and destroyed by mesangial cells and the result will be no or only a transient glomerular lesion. If however the function of the mesangial cells is disturbed the immune complexes remain on the basement membrane resulting in more severe damage to the membrane with ensuing glomerulonephritis (13). According to this hypothesis one could imagine how an infection via bacterial or viral metabolic products temporarily poisons the mesangial cells with resulting "febrile proteinuria". Recently it has been shown in experiments on rabbits that tubular damage as well may be caused by antigen-antibody complexes (10).

Whether infections result in deposition of immune complexes on the glomerular and/or tubular basement membrane can be evaluated by means of immunofluorescent microscopy on kidney biopsies obtained from patients with infectious diseases. Ward and Kibukamusoke (21) demonstrated malarial antigen as well as immunoglobulins and complement in kidney biopsies from patients with quartan malaria and the nephrotic syndrome and Combes et al (4) demonstrated the deposition of Australia antigen, IgG and complement (C 3) in a granular pattern in the glomeruli of a patient with persistent Australia antigen in the blood and a membranous glomerulonephritis. Furthermore in patients with infected ventriculoatrial shunt for hydrocephalus and with glomerulonephritis immunofluorescent microscopy of kidney biopsy has demonstrated the deposition of immunoglobulins and complement in the glomeruli (19).

Before the days of immunofluorescent microscopy investigations clearly demonstrated structural and functional renal abnormalities in patients with infectious mononucleosis, hepatitis and pneumonia of different etiologies (7-14, 18).

The etiology in most cases of glomerulonephritis is unknown. On the basis of the above mentioned investigations including the results of the present study it seems reasonable to propose that infectious diseases of different etiologies might be of

pathogenetic importance in the development of glomerulopathies.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

X Decremental Nerve Conduction in Uremia

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Abstract In uremic patient the sensory conduction velocity was relatively more reduced in the wrist-elbow segment than in the digit-wrist segment of the median nerve when the nervous impulse was initiated by supramaximal stimuli delivered to sensory fibers in digit 1 or 3. The proximal segment became affected earlier in the course of progressive kidney insufficiency than the distal segment. These findings were also apparent from the longitudinal course in the individual patient, and it is suggested that the uremic intoxication of the axon membrane function lead to decremental impulse propagation.

In a recent investigation it was shown that action potentials become conducted with decrement when peripheral nerves in normal persons are subjected to ischemia (1). The sequence of changes in the nerve conduction during 30 min of ischemia was principally similar to that observed over months or years during progressive chronic renal failure (CRF) (10). This supports the hypothesis previously advanced (10) that, in addition to degenerative nerve lesions slowing of the nerve conduction in uremia also results from a toxic impairment of the axon membrane function. The latter is inferred from other biophysical observations indicating that the Na^+ - H^+ activated ATPase is inhibited by dialysable humoral factor(s) in uremic serum (13) that the intracellular sodium concentration is elevated (1, 2) and that the resting membrane potential difference is reduced (3, 5). The present report brings evidence indicating that uremic intoxication leads to a decremental nerve conduction in analogy with the ob-

servations during ischemia and in keeping with the prediction by Lorente de N6 and Condouros (8).

MATERIAL AND METHODS

A total of 67 patient with CRF was examined on one or more occasions. Their age ranged from 15 to 61 years. The kidney function was expressed as the average 24-hour endogenous creatinine clearance (C_{cr} ml/min) for three consecutive day and corrected to body surface area of 1.73 m^2 .

The analysis was confined to the distal and proximal segments of sensory fibers in the median nerve hereby avoiding two sources of error: (a) The conduction pattern resulting from generalized toxic membrane dysfunction might become obscured by degenerative lesions, such as most prominent in the legs and predominantly affect the most distal part of the nerve. (b) If motor nerves an increase in the distal motor latency might reflect slowing of the conduction locality the terminal segment as well as an increased delay over the motor end plate, whereas the conduction velocity in more proximal segment is only determined by the latency difference between two sites of stimulation.

Sensory action potentials were evoked by supramaximal stimulation of the nerve in digit 1 ($n=14$) or digit 3 ($n=85$) and recorded ambidextrously through needle electrodes placed close to the nerve at the wrist and the elbow. (4) The action potentials were displayed on 3-channel electromyograph (DISA). The temperature near the nerve was about 35°C at both wrist and elbow. Other details about the procedure and mean values in normal material with the same age and sex distribution has been reported previously (9).

RESULTS

The regression (least square method) between conduction velocities in the digit-wrist (1) and the wrist-elbow (X) segments had a slope of 0.55 and

Part of this study was presented at the VIII International Congress of Electroencephalography and Clinical Neurophysiology Marseilles, 1973 (11).

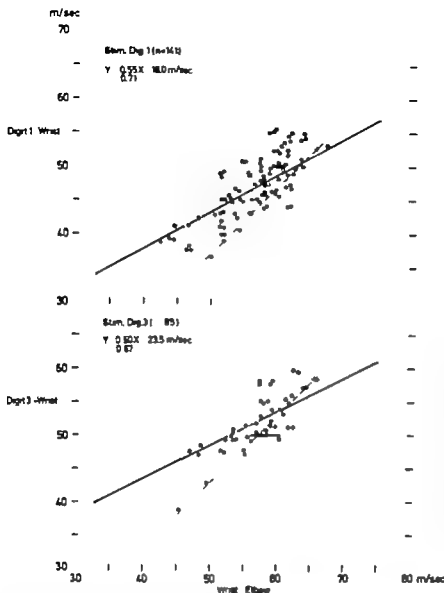


Fig. 1 Sensory conduction velocity in the distal (Y-axis) and proximal (X-axis) segments of the median nerve. ● = CRF patients, ○ = patients after renal transplantation. The equal-ranges illustrate the 95% of variation of the two variables in 48 normal subjects. Excluding measurements performed after renal transplantation the regression equations were: $Y = 0.49X + 17.9$ ($r = 0.68$) (digit 1) and $Y = 0.47X + 24.2$ ($r = 0.65$) (digit 3). $-\Delta Y = \Delta X$

0.50 $r = 0.71$ and 0.67 $p < 0.001$ (digits 1 and 3 respectively). The distribution of data around the line $\Delta Y = \Delta X$ (stippled in Fig. 1) differed significantly from the normal ($p < 0.005$) indicating that the slowing of the conduction velocity was more pronounced in the proximal than in the distal segment. This was also true when expressed as the percentage deviation from the normal mean value which was higher in the proximal segment.

When the conduction velocities (stim. digit 1 Fig. 2) were adjusted for the normal age variation ($\Delta V = m/sec$) and related to the kidney function ($\log C_{Cr}$) the intersection between the regression line and the lower 95% limit of normal variation (arrows in Fig. 2) corresponded to a creatinine

clearance of 3 and 7 ml/min/1.73 m² in the distal and proximal segments respectively. This indicates that the slowing of the nerve conduction occurred earlier in the course of progressive kidney insufficiency in the proximal segment.

The same pattern was demonstrable in longitudinal studies in individual patients followed for months or years until renal transplantation or death. Fig. 3 shows the course in one patient who developed severe neuropathy in terminal renal failure the same action potential was conducted at a slower rate in the proximal than in the distal segment. The ratio returned to normal several months after a successful renal transplantation. For comparison Fig. 4 shows the

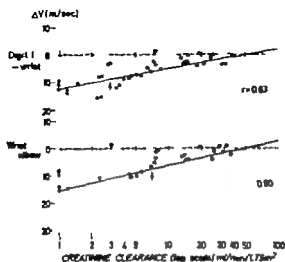


Fig 2 Sensory conduction velocity in distal and proximal segments of the median nerve related to the kidney function (log scale) in patients who have not been subjected to regular dialysis treatment. The observation were expressed as the deviation (ΔV m/sec) from the age-adjusted mean value in normal subjects. Shaded area=95% range of normal variation ($\pm 2 S_{95}$ for the regression, conduction velocity on age) in 48 normal subjects. Arrows=the C_{cr} level at which 50% of patient can be expected to show pathological conduction velocities. The regression equations were: $Y = 7.1X - 12.5$ $r=0.63$ (digit 1-wrist) and $Y = 9.1X - 15.4$ $r=0.60$ (wrist-elbow).

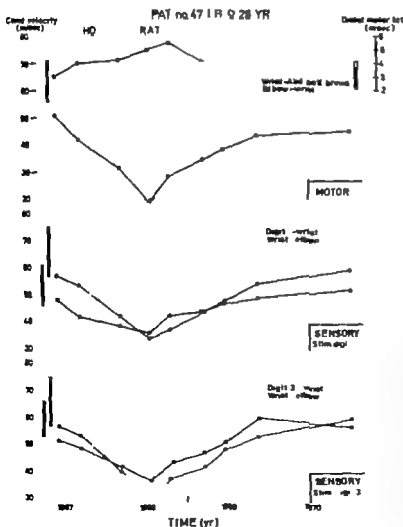


Fig 3 Longitudinal course of the motor and sensory conduction velocity in the median nerve before and after transplantation (R.A.T. full vertical line). H.D. (stippled vertical line)=start of regular hemodialysis. White and black vertical bars (± 2 S.D.) in normal subjects. Note the intersection of the curves for distal and proximal sensory conduction velocity.

slowing of the sensory conduction velocity in proximal and distal segments recorded during 30 min of ischemia in a normal person. A similar course is apparent

DISCUSSION

These findings cannot be explained by differences in temperature proximally and distally. Furthermore they are incompatible with an ascending degenerative nerve lesion, the commonly accepted pathological picture in uremia (6). Due to the increase in the temporal dispersion of the compound action potential in uremia (10) the first positive peak to which the latencies were measured may not represent the same fibers at the wrist and elbow, or from one examination to another. However by increasing temporal dispersion the first peak probably comes to represent slightly faster conducting fibers and more so the longer the conduction distance (4). Hence the different rate of slowing of the nerve conduction in distal and proximal segments may in fact have been underestimated. Studies in uremic patients comparable to the present one are not available from the literature. Kemble (7) reported that the index between the proximal and the distal conduction velocity was the same in normal subjects as in patients with uremic neuropathy. However he stimulated the nerve at two different levels and hence his data do not account for a possible slowing of the rate of impulse propagation in subsequent segments along the nerve.

The present results appear compatible with the hypothesis of a decremental nerve conduction reinstated and extensively discussed by Lorente de N6 and Condouin (8). This implies that the conduction velocity decreases as a function of the severity of the axon membrane dysfunction and of the length of the nerve segment through which the action potential is conducted. Both requirements seem fulfilled since, as previously discussed (10), the impairment of the membrane function is related to the severity of the uremic intoxication in all probability resulting in a reduction of the resting membrane potential difference (3, 5). The uremic intoxication therefore may become included among the factors enumerated by Lorente de N6 and Condouin (8) which can lead to decremental nerve conduction.

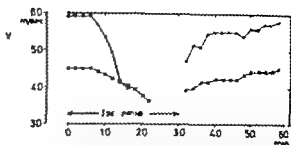


Fig. 4. The course of the sensory conduction velocity in the digit-wrist (\times) and the wrist-elbow (\circ) segment of the median nerve during and after 30 min of ischemia produced by inflation of a cuff placed around the upper arm to 200 mmHg. Normal subject male 41 year old.

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RENAL FAILURE ASSOCIATED WITH GENTAMICIN THERAPY

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Abstract A report is given on 6 cases of impaired renal function possibly caused by treatment with gentamicin. All patients had normal renal function before initiation of treatment. Two patients underwent peritoneal dialysis before recovery. In all patients renal function returned to almost normal level within 150 days after withdrawal of gentamicin. The importance of an individualized dosage of gentamicin related to body weight and renal function as well as frequent control of renal parameters and antibiotic serum concentration during treatment is emphasized.

Gentamicin is the newest antibiotic of established clinical importance among the aminoglycosides, which also comprise streptomycin and kanamycin.

All aminoglycosides are known to be potentially nephrotoxic. As regards gentamicin this was primarily demonstrated through animal experiments (1-5). The first experiences from the human clinic based on large patient materials showed a moderate impairment of the renal function in about 5% of the treated cases (3-11). Later reports have however described severe impairment of the renal function in connection with gentamicin therapy (9-13-22). In a nephrologic department 6 patients have within one year been treated for nephropathy in which gentamicin must be presumed to occupy a central place in the etiology. With a view to the extensive and increasing use of gentamicin publication of these cases is found justified.

CASE HISTORIES

Case 1

A 57-year-old previously healthy man was admitted for Staph aureus bacteremia. Treatment was instituted with penicillin and streptomycin after 6 days replaced by gentamicin, 80 mg three times daily methicillin, 8 g four times daily and folic acid, 500 mg three times daily. Serum creatinine and urography were at this time normal, but there was intermittent moderate proteinuria. The patient was at no time in shock.

During gentamicin therapy renal function became impaired and increasing proteinuria developed. After stopping of the treatment serum creatinine returned to normal level and proteinuria subsided. Vestibular examination showed almost discontinued vestibular function, which was fully restored 1 month later (Fig. 1 Tables I and II).

Case 2

A 54-year-old previously healthy man with Staph aureus septicemia, which caused transient shock without any influence on the normal renal function. Treatment was instituted with penicillin and streptomycin, after 4 days replaced by gentamicin, 80 mg three times daily and methicillin, 8 g 4 times daily.

During gentamicin therapy renal function became impaired and the patient developed oliguria and proteinuria. Microscopy of the urine sediment showed many erythrocytes, leucocytes, granular cylinders and erythrocyte cylinders.

Renal biopsy revealed pronounced tubulo-interstitial changes. In places the tubular epithelium was necrotic in places in state of regeneration and the tubular lumen contained protein and RBC cylinders, while the glomeruli were normal (sigs. II Hentzen).

After two dialyses the renal function had improved and serum creatinine returned to normal level. Gentamicin was still measurable in serum 4 days after withdrawal. Urography performed before discharge was normal (Fig. 2, Tables I and II).

Case 3

A 72-year-old man developed wound infection with Staph aureus after operation for varicose crumens, and treatment was instituted with gentamicin, 80 mg three times daily.

During gentamicin therapy the renal function decreased, but after withdrawal of the drug serum creatinine returned to normal level. The patient was at no time in shock. Urography before discharge was normal (Tables I and II).

Case 4

A 67-year-old man was admitted for cancer maxillary operation. Due to complicating pneumonia, treatment was instituted with erythromycin, after 4 days replaced by gentamicin 80 mg three times daily. On the first

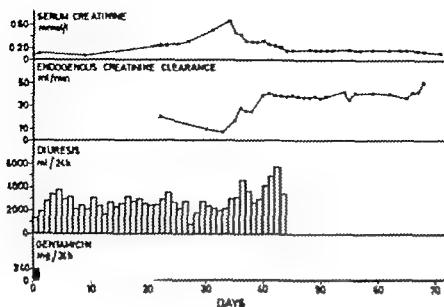


Fig. 1 Case 7. Serum creatinine, creatinine clearance, diuresis and gentamicin dosage.

days the treatment was supplemented by polymyxin 50 mg three times daily. The renal function was normal at the beginning of the treatment, and the patient was at no time in shock.

During gentamicin therapy the renal function decreased and dialysis became necessary.

About 3 months after withdrawal of gentamicin serum creatinine had returned to normal level. Three weeks after withdrawal, gentamicin was still measurable in serum. The serum concentration of polymyxin were all on a therapeutic level. I urography before discharge was normal (Tables I and II).

Case 5
A 75-year-old woman was admitted for treatment of colitis icterosa. The renal function was normal. Due to colon perforation with peritonitis laparotomy with ileostomy and colectomy was carried out. Postoperatively the patient was treated with streptomycin and

penicillin for 6 days, then the treatment was changed to gentamicin 80 mg three times daily. During the gentamicin therapy the renal function decreased, but after withdrawal of the drug it improved again. Before the renal function was fully restored the patient developed a *Candida albicans* septicemia, and she died shortly after (Tables I and II).

Case 6

A 55-year-old woman with evolving AV block was hospitalized for pacemaker implantation. The patient was cardially well compensated, and the renal function was normal. Due to infection round the implanted pacemaker the patient was treated with gentamicin 80 mg three times daily in three periods of 4, 8 and 19 days, at intervals of 10 and 12 days respectively. During the last period of treatment the renal function decreased, but after withdrawal of gentamicin serum creatinine slowly returned to normal level and the proteinuria

Table I. Changes in renal function during treatment with gentamicin

Case no.	Serum creatinine (mmol/l) (normal <0.13)		Creatinine clearance (ml/min)		Proteinuria (g/24 h)		Duration of treatment (d)	
	Before treatment	Maximum during treatment	Before treatment	Maximum during treatment	Before treatment	Maximum during treatment	Until renal function decreases	Total
1	0.09	0.68	7.9		III	4.0	14	22
	0.1	1.58	0.3		0.0	1.6	12	17
3	0.11	0.29	33.0		0.0		12	21
4	III	1.32	1.6		0.0		8	11
5	0.07	0.41	3.6		0.0	1.0	16	25
6	0.07	0.35	81.0	9.3	0.0	2.0	10	19

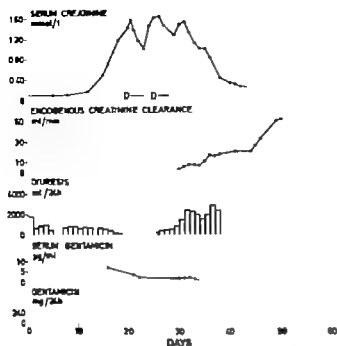


Fig 2 Case 2. Serum creatinine, creatinine clearance, serum gentamicin and gentamicin dosage. D=peritoneal dialysis.

stopped. I urography before discharge was normal (Tables I and II).

Gentamicin was in all cases administered intramuscularly and the serum levels were measured immediately before an injection (predose level). All patients had normal liver function prior to and during treatment.

DISCUSSION

All the presented patients developed nephropathy in association with gentamicin therapy. It is, however, difficult to evaluate clinically the nephrotoxicity of gentamicin in these patients who were often in a very poor condition and in whom fac-

tors like septicemia, hypotension or other medical treatment may have caused or contributed to the impaired renal function.

The following, however, supports the suspicion of gentamicin-induced renal damage: 1) In all patients the drop in renal function occurred some time after the acute stage of disease including BP fall and septicemia. 2) The renal function was normal at the beginning of gentamicin therapy and only started to decrease after 8–14 days of treatment. 3) The period of remission was extremely prolonged in all the patients as compared with uncomplicated shock kidneys, which are in most

Table II. Dosage of gentamicin, duration of proteinuria and renal impairment

Case no.	B. wt. (kg)	Gentamicin dosage (mg/kg/24 h)	Maximum serum predose level (µg/ml)	Oliguria	Duration of proteinuria after withdrawal of gentamicin (d.)	Duration of raised serum creatinine after withdrawal of gentamicin (d.)	Affection of tubular organ
1	56	4.5	—	No	12	50	Yes
2	65	3.7	—	Yes	38	65	H
3	78	3.0	6.2	No	—	37	No
4	65	3.1	>10.0	Yes	365	90	H
5	45	5.0	>10.0	N	>14	>14	
6	53	4.5	1.5	N	27	150	No

cases more quickly restored. 4) The renal biopsy revealed severe tubular damages, but unaffected glomeruli. Similar histological renal changes have been demonstrated by animal toxicologic investigations with gentamicin (1, 5, 10, 21).

However, several of the patients have simultaneously with gentamicin received other potentially nephrotoxic drugs. Thus cases 1 and 2 were treated with methicillin, which is known occasionally to cause nephropathy, probably through an allergic mechanism (17). Case 4 received polymyxin, which also belongs to the potentially nephrotoxic antibiotics (17).

Animal experiments have shown that dogs are particularly sensitive to the nephrotoxic properties of gentamicin as administration of ~ 3 times the human therapeutic dose causes degenerative changes in the tubular epithelium, and larger doses produce tubular necroses, while glomeruli are unaffected (1).

After administration of large doses of gentamicin similar renal changes have been described in rats, cats and monkeys (5, 10, 21). In all the experimental animals the cause of death was renal insufficiency and uremia.

Animal experiments have proved that there is a certain although rough connection between the dose mg/kg/24 h and the frequency of nephropathy (1, 5, 21).

The pathogenesis of gentamicin nephropathy is to be merely toxic damage of the tubules. As no justification for an allergic genesis has been found (4).

In the human medical clinic Falco et al. (3) state the frequency of impaired renal function during gentamicin therapy to be about 10% judged from measurements of blood urea. The renal damages found were slight and reversible. No correlation was found to exist between total dose or the duration of the treatment and the frequency or degree of renal damage.

Hewitt (9) has reported 3 cases of gentamicin-induced renal impairment, and Kahn and Stein (13) have described a case of non-oliguric renal insufficiency in which the renal function was only partly restored 5 months after the end of the treatment.

Wilfert et al. (22) have reported gentamicin-related reduction of renal function in 4 patients. In all cases the renal function was fully restored.

Opitz et al. (16) refer to 10 cases of renal im-

pairment in a group of 16 patients who were treated simultaneously with gentamicin and cephalothin. All patients had normal renal function at the start of the treatment. Hewitt (9), Schultze et al. (19) and Wilfert et al. (22) have also described cases of renal insufficiency occurring during simultaneous treatment with gentamicin and cephalothin. These and more recent cases (2, 4, 15) imply that this combined treatment involves a special risk of renal impairment.

Summing up, it must be admitted that there is no definite evidence of the pathogenic importance of gentamicin in some of the patients covered by this study, but based on the case records, the histology of the renal biopsy and the reviewed animal experiments and clinical reports we find that this is the most probable cause in the cases described.

By and large the observed cases of renal impairment were reversible, serum creatinine being normalized in 5 patients after about 37–150 days (average about 80 days). The long periods of remission should be ascribed to the severe tubular damages. As no comparable clearance measurements were made before and after gentamicin therapy, it is not possible definitely to evaluate the possibility of permanent reduction of renal function in the patients here concerned.

Gentamicin is almost exclusively excreted renally by glomerular filtration (1, 7). In 2 of the present patients gentamicin was still measurable in serum more than 3 weeks after the last administration. This slow elimination may—besides the reduced renal function and the protein binding of gentamicin (1)—be ascribed to the fact that if the time of diffusion is sufficiently long, gentamicin is also to some extent capable of diffusing into the intracellular space (8). The diffusion back again of this intracellularly distributed gentamicin is undoubtedly a very slow process. In peritoneal dialysis the elimination is insignificant (6, 20). Therefore, if reduction of the gentamicin content of the organism in patients with kidney insufficiency is indicated, hemodialysis is the only efficient method (18).

The magnificent antibacterial properties of gentamicin have justified its extensive clinical use. The potential nephrotoxicity should however always be borne in mind. The present case histories emphasize the risk of standardized treatment with gentamicin. If oto- and nephrotoxic side-effects

are to be avoided. It is of the greatest importance to adapt and individualize the dosage not only according to body weight but also to renal function, which in ill pts. before institution of the treatment determination of serum creatinine, creatinine clearance, examination for proteinuria and microscopy of the urine sediment. Evaluation of the glomerular filtration exclusively based on serum creatinine is not adequate. In the first place the correlation between serum creatinine and creatinine clearance is not linear and secondly the glomerular filtration may vary considerably according to age and sex without being reflected in serum creatinine (14). Guidance regarding individualized dosage in patients with reduced renal function is given by Gingell and Waterworth (7). During the treatment regular control of serum creatinine and creatinine clearance is necessary and at the least sign of decreasing renal function, which may reasonably be ascribed to gentamicin the treatment should be immediately discontinued. Determinations of serum concentrations are most helpful in the search for the optimal dosage and are considered necessary all through the treatment of patients whose renal function is already impaired.

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SERUM URIC ACID LEVELS IN WOMEN

An Epidemiological Survey with Special Reference to Women with High Serum Uric Acid Values

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Abstract Women in the age strata 38, 46, 50, 54 and 60, 1462 women in all, were submitted to a population study in Göteborg, Sweden. Determination of serum uric acid levels was included in the study. The mean values of serum uric acid increased from 3.6 mg/100 ml in women aged 38 to 4.4 mg/100 ml in women aged 60. The rise in serum uric acid with age seemed to be unrelated to the menopause as no differences were found between premenopausal and postmenopausal women of the same age. None of the 1462 women studied was found to suffer from clinical gout. When comparing women with high serum uric acid values and women in the general population of corresponding ages no significant differences were found for joint complaints, serum creatinine, history of cystitis and/or pyelonephritis, history of renal calculi, alcohol consumption or socio-economic status. Women on saluretic diuretics had significantly raised serum uric acid values compared to the general population of women. The urine concentrating capacity was decreased in hyperuricemic women both in those who took and in those who did not take saluretic diuretics. Women with high serum uric acid values had significantly higher values for body weight, subscapular skinfold and Hb. Serum uric acid was raised in women with arterial hypertension, women with history suggesting angina pectoris and women with ECG changes suggesting ischemic heart disease. However these differences were probably caused by an overrepresentation of women on saluretic diuretics in these groups.

Since the middle of the 18th century it has been known that there is an association between high uric acid levels and clinical gout (27). However as found from previous population studies, all people with high serum uric acid levels do not develop clinical gout (16, 21). A possible association between high serum uric acid values and ischemic heart disease has also been discussed

though previous population studies do not definitely support such an association (15, 22). An association has been found between high arterial BP and high serum uric acid levels (9, 17). Furthermore saluretic diuretics are known to raise serum uric acid values (10).

The present paper presents further data on these matters. Serum uric acid levels in a population sample of women are presented. Some characteristics of women with high serum uric acid values are given. Three groups of women participating in the population study are discussed separately: those on saluretic diuretics, those with high BP who are not on saluretic diuretics and those with symptoms or ECG changes suggestive of ischemic heart disease.

MATERIAL AND METHODS

A population study of women in Göteborg, Sweden, was performed in 1968-69 (7). Women in the age strata 38, 46, 50, 54 and 60 were examined (Table I), altogether 1462 women (participation rate 90.1%). Women born on dates which were even multiples of six were called for the study (7). In this way the population sample was considered to be representative of the female population in Göteborg in the age strata studied. The survey was performed for the most part during 12-month period. In this way the influences of age differences within each age group and of seasonal variations were reduced as far as possible.

Blood sampling was performed in the morning after overnight fasting (7). Serum uric acid levels were determined manually by an enzymatic method essentially according to Praetorius (26) using Urikase L (Larvens Kemiske Fabrik, Denmark) on deep-frozen sera (-20°C). The day-to-day variation ($S.D. \times 100/\text{mean}$) was found to be 4.1%. Values within the upper five centiles of

Table 1 Serum uric acid levels (mg/100 ml) in a population sample of women in Göteborg 1968-69

Age (y)	n	Mean value	S.D.	S.E.	Median value	Range
38	368*	3.6	1	0.06	3.5	0.9-9.0
46	479*	3.8	1.1	0.05	3.7	0. - 8.0
50	396*	4.1	1.2	0.06	3.9	1.0-11.6
54	176*	4.3	1.4	0.11	4.0	1.6-10.9
60	111	4.4	1.5	0.17	4.2	0.6-9.0

* 4 data missing, data missing.

the population sample in the different age strata were defined as high values (hyperuricaemia).

Twenty-nine women in the population sample reported history of angina pectoris (6) as defined according to Rose (28). ECG changes implicating Minnesota Codes 1-1, 4-1, 5-1, 2 (in the absence of 3-1), 6-1, 7-1 (3-8) were defined as coronary ECG and recorded in 23 women participating in the population study (6).

Statistical methods: Conventional methods were used for calculation of median value, mean value, standard deviation (S.D.) and standard error of the mean (S.E.). The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation (6) hypothesizing that the characteristics studied were more common in women with high serum uric acid values (one-tailed test). Significance of differences between mean values was estimated using Student's *t*-test.

RESULTS

Serum uric acid levels in the female population

Table 1 shows mean values, S.D., S.E., median values and ranges of serum uric acid values in the different age strata studied. There is a rise with age, from 3.6 mg/100 ml in women aged 38 to 4.4 mg/100 ml in women aged 60. Fig. 1 shows the distributions of serum uric acid values. The distribution is normal in women aged 38 except for a few extremely high values, while the distributions of women aged 46-60 are somewhat skewed

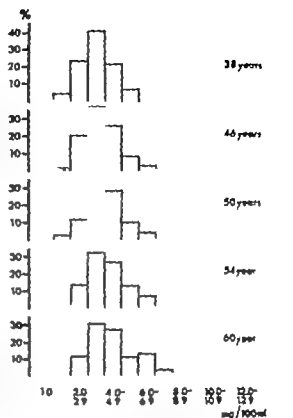


Fig. 1 Distributions of serum uric acid values in a population sample of women in Göteborg 1968-69

to the right with mean values higher than median values.

Serum uric acid levels were found to rise with age also when women of similar body weight in the various age strata were compared (Table II).

Comparison between pre- and postmenopausal women: In Table III the women participating in the population study are subdivided into pre- and postmenopausal women. No differences were found in serum uric acid levels between pre- and postmenopausal women of the same age. Women with surgical menopause are not included in the Table as it could often not be established whether

Table II Serum uric acid levels (mg/100 ml) in women of similar body weight (kg)

Age (y)	B wt. 55.0-59.9			III wt. 60.0-64.9			B wt. 65.0-69.9		
	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
38	111	3.2	0.9	76	3.5	1.1	49	3.7	1.0
46	96	3.7	1.1	114	3.6	1.0	62	3.8	0.9
50	66	3.9	1.3	90	4.0	1.2	77	4.1	1.0
54	25	3.7	1.3	39	4.3	1.1	79	4.5	1.3
60	13	3.8	0.5	15	4.2	1.6	16	4.8	1.5

Table III. Serum uric acid levels (mg/100 ml) in pre- and postmenopausal women in the population sample

Age (y)	Premenopausal		Postmenopausal ^a		Significance of difference
	Mean	S.D.	Mean	S.D.	
38	3.7	3.6	1.2	—	
46	3.7	3.8	1.1	3.3	N.S.
50	4.1	4.1	1.4	4.0	N.S.
54	4.5	4.5	1.6	4.2	N.S.
60	—	—	1.6	4.4	

No menstruation during the last 6 months; women with surgical menopause not included

both ovaries had been removed in women who had been subjected to hysterectomy. There was no correlation between serum uric acid levels and time since last menstruation either as will be seen from Table IV which shows serum uric acid values in postmenopausal women according to time since last menstruation.

Characteristic of women with high serum uric acid values

Women with serum uric acid values within the upper five centiles in the different age strata (Table V) have been defined as hyperuricaemic. Some characteristics of them (different age strata taken together) are shown in Table VI.

Clinical gout. None of the participants in the population study had a history of clinical gout.

Joint complaints. The participants were asked for finger joint complaints. Finger joint complaints were not more common in hyperuricaemic women than in women in the population sample irrespective of whether a history of or the prevalence of finger joint complaints at the time of the population study were taken into consideration.

Table V. Borderline values (mg/100 ml) for hyperuricaemic women

Age (y)	Borderline upper 5 centiles
38	4.5
46	5.8
50	6.1
54	6.7
60	7.0

Arterial hypertension and use of saluretic diuretics. Serum uric acid in hypertensive and non-hypertensive women and in women who took and those who did not take saluretic diuretics will be dealt with below. As may be seen from Table VI, a history of arterial hypertension was about 3 times as common and the use of saluretic diuretics about 4 times as common in the hyperuricaemic women as in the total population sample.

Kidney diseases. Proteinuria was significantly more common in hyperuricaemic women. The difference disappeared if women on saluretic diuretics were excluded. Thus 37% of the hyperuricaemic women who took saluretic diuretics had proteinuria compared to 7% of those who did not take and to 6% of women in the total population sample.

The urine concentrating capacity was decreased in hyperuricaemic women. A decrease (<600 mOsm/kg H₂O after 13 hours thirst) was noted in 36% of hyperuricaemic women who took saluretic diuretics but there was also a difference between hyperuricaemic women who did not take saluretic diuretics (22%) and the women in the total population sample (1%, $p < 0.05$).

A history of renal calculi (doctor's diagnosis) was as common in the total population sample as in the hyperuricaemic women. There were no sig-

Table IV. Serum uric acid levels (mg/100 ml) in postmenopausal women according to time since last menstruation

Age (y)	Time since last menstruation											
	2-11 mo		1-4 y		5-9 y		≥10 y					
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D		
46	28	3.7	1.0	33	3.6	1.1	111	4.2	0.9	5	3.6	0.6
50	51	4.0	1.4	116	4.0	1.3	32	3.9	0.8	16	3.8	1.1
54	17	3.9	1.4	60	4.2	1.3	62	4.4	1.7	26	4.3	1.1
60							18	4.4	1.9	60	4.4	1.4

Table VI Some characteristics of hyperuricaemic women compared to the total population sample

Variable studied	Hyperuricaemic women (%)	Total population sample (%)	Significance of difference
Clinical gout	0	0	N.S.
History of finger joint complaints	16.0	14.5	N.S.
Prevalence of finger joint complaints causing a visit to a doctor	2.7	6.2	N.S.
History of arterial hypertension*	24.0	8.6	$p < 0.001$
Use of saluretic diuretics	21.3	5.0	$p < 0.001$
Proteinuria	13.7	6.3	$p < 0.01$
Urine concentrating capacity $< 600 \text{ mOsm/kg H}_2\text{O}^b$	23.0	11.8	$p < 0.001$
History of renal calculi	5.3	5.3	N.S.
History of cystitis and/or pyelonephritis	38.7	40.8	N.S.
History of pyelonephritis	9.3	6.5	N.S.
Beer several times a week	28.4	26.5	N.S.
Wine several times a week	2.7	4.9	N.S.
Spirits several times a week	0	1.2	N.S.
High socio-economic status*	1.4	6.0	N.S.
High socio-economic status of husband	13.6	13.8	N.S.

During pregnancy only not included.

After 13 hours' thirst.

E.g. large scale employers and officials of high or intermediate rank (%).

nificant differences concerning history of cystitis and/or pyelonephritis.

Alcohol consumption No significant differences were found between hyperuricaemic women and women in the total population sample when comparing the numbers of women who consumed alcohol several times a week.

Socio-economic status There were no significant differences in socio-economic status. If any thing, the socio-economic status tended to be lower in hyperuricaemic women. No differences were found in socio-economic status concerning the husbands of the women either.

Anthropometric data Table VII shows some anthropometric data on hyperuricaemic women compared to the total population sample in the different age strata. There were no significant differences in body length. Body weight tended to be higher in hyperuricaemic women in all the age strata studied. Significant differences were found for women aged 38 and 54. Similarly the subscapular skinfold was thicker in the hyperuricaemic women. Significant differences were found for women aged 38, 46, 54 and 60.

Haemoglobin The Hb values were found to be higher in the hyperuricaemic women than in the total population sample in the different age strata studied. Significant differences were found for women aged 38, 46 and 54 (Table VII).

Serum uric acid in women on saluretic diuretics

As shown in Table VI hyperuricaemic women were significantly more often on saluretic diuretics than the women in the total population sample. As seen from Table VIII participants on saluretic diuretics also had significantly higher serum uric acid levels than the total population sample in the different age strata studied.

Serum uric acid in women with arterial hypertension

Table IX shows serum uric acid levels in women who received saluretic diuretics because of arterial hypertension at the time of the population study and of women who had hypertension (systolic BP $\geq 160 \text{ mmHg}$ in sitting position) but who were not on saluretic diuretics at the time of the study. Women who took saluretic diuretics because of hypertension had significantly higher serum uric acid values in all the age strata studied while the values were similar in women with systolic BP $\geq 160 \text{ mmHg}$ who were not on saluretic diuretics and in the total population sample in the age strata studied.

*Serum uric acid in women with angina pectoris and coronary ECG**

As will be seen from Table X women in the population sample who had angina pectoris ac-

Table VII Anthropometric data and Hb levels of hyperuricaemic women compared to the total population sample

Hyperuricaemic women				Total population sample				Significance of difference
Age (y.)		Mean	S D		Mean	S D		
Body length (cm)								
38	19	164.3	5.9	372	164.6	5.7	N.S.	
46	22	162.3	5.8	431	163.5	7.5	N.S.	
50	20	163.4	4.8	398	163.5	5.5	N.S.	
54	9	166.1	9.7	180	162.6	5.4	N.S.	
60	5	160.6	6.4	81	162.0	5.1	N.S.	
Body weight (kg)								
38	19	74.1	22.3	372	63.4	11.1	$p < 0.001$	
46	22	66.1	12.1	431	63.0	9.8	N.S.	
50	20	69.2	11.9	398	66.2	11.1	N.S.	
54	9	81.1	17.1	180	66.0	12.5	$p < 0.001$	
60	5	68.4	11.8	81	66.1	10.9	N.S.	
Subscapular skinfold (mm)								
38	19	22.5	10.3	372	15.3	7.1	$p < 0.001$	
46	22	19.3	8.1	430	16.1	7.1	$p < 0.05$	
50	20	19.3	8.3	398	18.8	8.2	N.S.	
54	9	26.3	10.8	180	18.4	8.2	$p < 0.01$	
60	5	25.6	9.1	81	19.6	8.5	$p < 0.01$	
Hb (g/100 ml)								
38	19	14.0	0.97	372	13.5	1.02	$p < 0.05$	
46	22	14.0	1.00	431	13.5	1.15	$p < 0.05$	
50	20	13.8	1.27	398	13.7	1.10	N.S.	
54	9	14.7	1.02	180	13.8	0.99	$p < 0.01$	
60	5	14.5	0.77	81	13.8	0.85	N.S.	

cording to the definition used (mean age 51.3) had significantly higher serum uric acid values than a reference group of women of similar mean age (the participants aged 50). However when the women with angina pectoris were subdivided into those who took and those who did not take saluretic diuretics, the difference was found to be caused by those on saluretic diuretics only. Thus the mean value was found to be 4.2 mg/100 ml in the women with angina pectoris who did not take saluretic diuretics compared to 4.1 mg/100 ml

in the reference group. As shown in Table X women with "coronary ECG" (mean age 54.2) had significantly higher serum uric acid levels than a reference group of similar mean age (the participants aged 54). However this difference could also be shown to be caused by those in the "coronary ECG" group who took saluretic diuretics. If these women are excluded the mean value was found to be 4.3 mg/100 ml both in the group of women with coronary ECG and in the reference group.

Table VIII Serum uric acid values (mg/100 ml) of women on saluretic diuretics in the population sample and of the women in the total population sample

Age (y.)	On saluretic diuretics			Total population sample			Significance of difference
	Mean	S.D.		Mean	S.D.		
38	6	5.2	1.4	368	3.6	1	$p < 0.01$
46	11	4.6	1.3	429	3.8	1.1	$p < 0.05$
50	19	5.0	1.6	396	4.1	1.2	$p < 0.001$
54	21	5.6	2.0	176	4.3	1.4	$p < 0.001$
60	15	6.1	1.5	81	4.4	1.5	$p < 0.001$

Table IX. Serum uric acid values (mg/100 ml) of hypertensive women on saluretic diuretics in the population sample of women with systolic BP ≥ 160 mmHg at the population study examination who were not on saluretic diuretics and of the women in the total population sample

Age (y)	Hypertensives on saluretic diuretics			Systolic BP ≥ 160 not on saluretics			Total population sample		
	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
38	3	5.3	2.1	3	4.7 ^a	0.9	368	3.6	1.2
46	10	4.6	1.4	22	3.7 ^a	1.0	429	3.8	1.1
50	15	4.8	1.5	46	4.3 ^a	1.6	396	4.1	1.2
54	18	5.9 ^a	2.0	31	4.4 ^a	1.2	176	4.3	1.4
60	10	5.9 ^a	1.5	24	4.0 ^a	1.4	81	4.4	1.5

$p < 0.05$ $p < 0.01$ *** $p < 0.001$ significantly different from women in the general population.
Not significant.

DISCUSSION

The results presented in this paper derive from a population study of a representative sample of women in five age strata in a defined urban area. Due to the means of selection and the high participation rate the results are considered to be valid for the population studied.

The serum uric acid levels were found to rise with age in the present study which finding agrees with previous results elsewhere as reviewed by Neel (23) and as will be seen in Fig. 2 in which the present results are compared with those from some previous studies of women. The Göteborg values seem to have an intermediate position and agree most closely with those reported from Framingham, USA (16) Saloinen Finland (17) and Wexleydale UK (25). As determinations may be made on fresh or deep-frozen sera and different methods for determination of serum uric acid are used, owing to which some differences may be expected (11, 23, 24). It seems unwise to conclude

that serum uric acid levels are definitely different in women in different parts of the world.

Previous studies indicate that serum uric acid levels are stable in males from puberty onwards and about 1 mg/100 ml higher in men than in women until the time of the menopause of the women (23). This also agrees with the results of the present study and a previous population study of men in Göteborg. Mean serum uric acid level was found to be 5.3 mg/100 ml in 50-year-old men (32) compared to 4.1 mg/100 ml in 50-year-old women.

The rather constant finding in previous studies of a fairly steep rise in serum uric acid during the climacteric age but rather stable levels before this age (16, 17, 24, 25, 30) has led to the assumption that the rise in serum uric acid during the 5th and 6th decades is caused by the menopause. However, there seems to be no previous study in which pre- and postmenopausal women of the same age are compared. In the present study

Table X. Serum uric acid values (mg/100 ml) of participants in the population study with angina pectoris and 'coronary ECG' and in the total number of participants in the age strata 50 or 54

Whole group			On saluretics			Not on saluretics			Whole group		
n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.
<i>Angina pectoris</i>											
29	4.9 ^a	1.7	7	7.0 ^a	1.3	22	4.7 ^a	1.1	<i>Population sample aged 50</i>		
									396	4.1	1.2
<i>Coronary ECG</i>											
23	5.0 ^a	1.6	12	5.6	1.5	11	4.3 ^a	1.3	<i>Population sample aged 54</i>		
									176	4.3	1.4

$p < 0.05$ $p < 0.01$ *** $p < 0.001$ significantly different from the women in the general population.
Not significant.

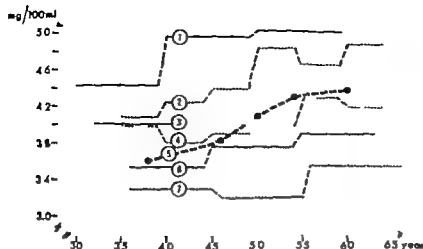


Fig 2 Means of serum uric acid levels in women in the present study and in some previous surveys. 1=New Zealand (12), 2=Tecumseh, USA (21), 3=Framingham, USA (16), 4=Finland (17), 5=the present study, 6=Walsleydale, UK, (25), 7=DDR (30).

age strata instead of age groups were used. In this way pre- and postmenopausal women of almost identical age could be compared and no differences were found between them. The rise in serum uric acid levels with age in women thus seems to be unrelated to the menopause.

The prevalence of clinical gout is lower in women than in men (16, 18, 24, 29). All hyperuricaemic subjects do not suffer from gout. Thus 3 of 17 women in Framingham who had serum uric acid values ≥ 7 mg/100 ml at a single examination had had clinical gout at the 12 year follow-up (16). In the present study none of the women was known to suffer from clinical gout. In view of previous studies of women this could be anticipated. In two studies in Japan none of about 14000 women was found to have gout (18, 29). There was no overrepresentation of women with joint complaints among those with high serum uric acid values in the present study either.

Saluretic diuretics are known to raise serum uric acid levels (10, 17, 31). A significant difference between those who took and those who did not take saluretic diuretics was also found in the present study. The differences were found to be about 1.0–1.5 mg/100 ml in the different age strata. These figures also agree with those found in a subsequent study of hypertensive participants (5) comparing placebo, chlorthalidone (a saluretic substance) and alprenolol (a β -receptor blocking agent). Serum uric acid levels were on an average 1.4 mg/100 ml higher on chlorthalidone than on placebo.

There seems to be an association between high serum uric acid levels and kidney disease (16, 17)

and between gout and renal stones (24, 33). In the present study no differences in serum creatinine levels or in history of cystitis and/or pyelonephritis were found between women with high serum uric acid values and the total population sample, nor was there any difference regarding history of renal calculi. There were very few women with badly impaired function as judged from serum creatinine values in the population sample. The results therefore do not exclude an association between high serum uric acid and kidney disease which seems to be present in subjects with severely impaired renal function (14).

Proteinuria was significantly more common in women with high serum uric acid values in the present study than in the total population sample. This disagrees with the results from Framingham where no significant difference was found between hyperuricaemic subjects and those in the total population sample (16). However the difference in the present series seems to be caused by overrepresentation of women on saluretic diuretics among those found to be hyperuricaemic and overrepresentation of women with proteinuria among those taking saluretic diuretics. When women on saluretic diuretics are excluded, no difference is found between hyperuricaemic women and women in the total population sample which is then in agreement with the results from Framingham, as the serum uric acid determinations in Framingham were carried out before the introduction of saluretic diuretics.

Tubular function measured as urine concentrating capacity was decreased in hyperuricaemic women in the present study. This difference is

obvious even when women on saluretic diuretics are excluded. It seems possible that tubular damage may cause hyperuricaemia or that hyperuricaemia may cause tubular damage. There seem to be no other reports from population studies on the association between serum uric acid and urine concentrating capacity.

Some investigators have noted an association between arterial hypertension and high serum uric acid levels (9, 17, 19, 30) while others have found no or at most a very weak such association (17, 15, 22). The study of Isomäki (17) though carried out on hospitalized subjects is of especial interest, as hypertensive subjects who were and who were not on saluretic diuretics were also studied separately. The differences in serum uric acid between hypertensive subjects and subjects in the general population were reduced when those on saluretic diuretics were excluded but the author concluded that saluretic diuretics could not explain all the difference. In the present study women with high serum uric acid values had significantly more often a history of arterial hypertension and hypertensive women had significantly higher serum uric acid values than women in the general population. However, this difference seemed to derive entirely from those hypertensives who took saluretic diuretics, as no differences were found when they were excluded.

A possible association between ischaemic heart disease and serum uric acid has been discussed (4). No association was found in Tecumseh, USA (22). In Framingham, USA an association was found between gout and ischaemic heart disease but not between high serum uric acid and ischaemic heart disease (15). Isomäki (17) reported from Finland an association between high serum uric acid and ischaemic heart disease (hospitalized subjects) which mostly disappeared when women on saluretic diuretics and women with high serum creatinine values were excluded. In the present study an association between ischaemic heart disease and high serum uric acid values was found both when studying women with a history suggesting angina pectoris and women with ECG changes suggesting ischaemic heart disease. However, many of the women classified in these two categories were on saluretic diuretics, and if these women were excluded no differences remained.

An association between serum uric acid and body weight has consistently been reported in

previous studies (2, 12, 16, 19, 24, 30). An association has also been reported between skinfold and serum uric acid (12) and between body weight and gout (16, 24). In the present study body length was similar in women with high serum uric acid and in the general population, while the values for body weight and subscapular skinfold were higher in the women with high serum uric acid. The differences were mostly statistically significant. They were not attributable to saluretic diuretics as women with high body weight and women in the general population did not differ in this respect.

The difference in subscapular skinfold may indicate that differences in body fat are at least partly responsible for the association between body weight and serum uric acid.

Like serum uric acid, body weight increased with age (Table VII). The rise in serum uric acid with age might thus be secondary to the rise in body weight with age and non-related to age per se. In order further to elucidate this question, women of similar body weight in the different age strata were compared as to serum uric acid values. As shown in Table II, serum uric acid levels rose with age in women of similar body weight. Thus at least part of the rise with age in serum uric acid levels was non-related to body weight and the rise with body weight non-related to age.

An association between Hb and serum uric acid has been reported in males (2, 1). One study noted an association in women also (2) while another failed to reveal such an association in women (12). Significant differences between women with high serum uric acid and the general population of women were found in three of five age strata studied in the present series.

One reason may be a higher rate of cell destruction in healthy subjects with a large red cell volume, which may be the case in polycythaemia and leukaemia (13). The difference in red cell mass between men and women (6) might possibly explain some of the sex difference in serum uric acid levels. Lower serum uric acid levels in women of premenopausal than of postmenopausal age and in men have been considered to be explained by the loss of cells occurring in the menstrual flow of premenopausal women (2). However, this is not in accordance with our findings, which did not indicate any difference in serum uric acid levels between premenopausal and postmenopausal women of similar age.

According to previous studies there is an association between high alcohol consumption and high serum uric acid values in men (1, 17, 31). There seems to be no study which has revealed such an association in women nor did the present one do so. The reason is probably the low alcohol consumption of women compared to men (6) and the rapid decrease in serum uric acid after the last intake of alcohol (20).

According to medical tradition it is said that gout is a disease of wealthy people. However, previous studies indicate that high serum uric acid levels are more common in subjects of low socio-economic status (1) and a similar tendency was noted in the present study although the difference was not statistically significant.

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PROGNOSIS IN SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Abstract The prognosis of 187 patients with severe chronic obstructive pulmonary disease has been evaluated by use of the life table method. All patients had FEV₁ less than 60% of VC and below 1.5 l. The observation time ranged from 4 years and 4 months to 7 years and 4 months. The cumulative survival rate in the whole material was 71% after 3 years and 47% after 6 years, results which are very close to the survival found by other authors. Calculations are presented of the survival of FEV₁ subgroups and of clinical functional groups separately and combined. These two classifications have a good correlation to survival, and it is demonstrated that the combination of functional group and FEV₁ subgroups adds more information regarding the prognosis than that obtained through FEV₁ alone. Among patients with earlier pulmonary tuberculosis the prognosis was related only to the degree of pulmonary obstruction. The expected increase in lethality with advancing age was demonstrated, but the mortality was relatively higher in the younger age group when compared to the general population of the same age. The prognosis of patients with cor pulmonale was considerably worse than of patients without cor pulmonale even when the higher degree of severe airway obstruction was considered.

The prognosis of chronic obstructive pulmonary disease has been shown to be related to several features of the disease, such as vital capacity (VC) (3, 4, 5, 22), maximal breathing capacity (1, 3, 4, 16), forced expiratory volume in first second (FEV₁) or mid-expiratory flow rate (5, 14, 20, 22, 24), residual volume, pulmonary diffusing capacity for CO (1, 3, 5, 10, 20), radiological evidence of emphysema (23), cardiac disease, especially cor pulmonale (3, 5, 10, 14, 20, 22, 23), clinical functional group (5, 13, 17, 19), arterial oxygen saturation and CO₂ tension (4, 10, 14, 20, 23, 25), polycythaemia (19), early age at onset of disease or symptoms (16, 18, 1, 23), residence at high altitude (22) and weight loss (3, 4, 26).

Several of these features are interrelated and associated with expiratory slowing (5, 26) and

when their relative value for prognosis was examined (5). It turned out that if FEV₁ was the same then the prediction of survival was improved mainly by resting heart rate (higher heart rate lower survival), overall assessment of functional class, history of edema and to a smaller extent by ECG signs of cor pulmonale. Clinical evaluation of functional test, improvement in forced expiratory volume with isoproterenol and by albumin/globulin ratio. It seems also that the prediction of the prognosis is improved by consideration of the decrease in FEV₁ over a year period (5). The prognosis in relation to FEV₁ seems in different studies to be nearly the same and patients with a FEV₁ above 1.5 l have a prognosis of survival not much different from a normal population of the same age and sex (3, 11, 22). The FEV₁ is the parameter best correlated to the clinical functional evaluation (7, 8, 9).

The present report describes the prognosis of survival for patients with severe chronic obstructive pulmonary disease in relation to the value of FEV₁ and to the clinical functional group separately and combined.

MATERIAL AND METHODS

Patients from Medical Departments B, C and P Bispebjerg Hospital, Copenhagen, were incorporated in this study if they had had pulmonary function test performed in the period Jan 1st 1964 - Dec 31st 1966. Included for further study were patients who had a FEV₁ below 1.5 l if this was less than 60% of VC. Patients with pulmonary carcinoma or other cancers present at the time of the pulmonary functional test were excluded.

Evaluation of clinical functional group was made according to the classification used by Jensen et al (13). Although it might have been desirable to use more detailed classification, this was not possible with the information obtainable from the patient files.

Table 1 Age and sex distribution FEV₁ and clinical functional group of the patients

No. of pat.	Age (y)	Sex	FEV (l/sec)				Functional group			Tub. pat.
			0.0-0.6	0.7-0.9	1.0-1.2	1.3-1.5	A	B	C	
1	0-29	♂	0	1	0	0	1	0	0	0
0		♀	0	0	0	0	0	0	0	
2	30-39	♂	0	1	0	1	1	1	0	4
6		♀	1	2	1	2	2	3	1	
20	40-49	♂	2	7	7	4	4	15	1	18
13		♀	1	5	7	0	5	11	2	
47	50-59	♂	9	15	16	7	6	31	9	29
23		♀	4	8	9	2	3	18	1	
46	60-69	♂	6	27	15	8	11	33	11	20
11		♀	6	4	0	1	2	4	1	
7	70-79	♂	2	5	0	0	0	2	5	0
1		♀	0	0	1	0	0	0	0	

The study was terminated on April 1st 1971. The longest observation period was 7 years and 4 months and the shortest 4 years and 4 months. To get the maximum information, the life table method was used for calculation of the cumulative survival rate (survival).

The standard errors of the 1st, 3rd and 5th years accumulated survival rates are worked out from the tables of Ederer (11) which are computed from an approximation to Greenwood's formula. Standard error values based on less than 50 cases are not shown, because rates based on less than 30 cases are too unreliable to be of value. The fate of all patients by the 1 of April 1971 was known and the date of death also known for all patients dying during the observation period.

RESULTS

One hundred and eighty-seven patients, of whom 54 were women, were selected in the way described. Table 1 shows the age and sex distribution in relation to FEV₁ and clinical functional group. The mean age for the whole material is 57 years (range 20-79) and the mean FEV₁ 0.9 l. S.D. 0.25 l. (range 0.35-1.50).

The survival in the whole material was 71% after 3 years, 63% after 4, 57% after 5 and 47% after 6 years as seen in Table II. The survival for subgroups according to FEV₁ is seen in Fig. 1. The 6-year survival of patients with an initial value of FEV₁ below 0.6 l. is 22%. 37% for those with FEV₁ 0.7-0.9 l., 53% for those with FEV₁ 1.0-1.2 l. and 78% for those with FEV₁ 1.3-1.5 l. In a "normal population" from Copenhagen of the same age and sex as the 187 patients 87% survived for 6 years (Statistical Yearbook 1967).

Among men below 60, 46% were dead after 11 years of observation; this percentage is more than 6 times as high as would be expected in a "normal population" of the same age in Copenhagen. Out of 63 men above 60 years, 66% died during 6 years of observation. This is 2.2 times more than expected (Table III).

Patients with earlier active tuberculosis were included in the study if the lung function test was as described. After 6 years 60% of the patients with earlier active tuberculosis of the lungs are still alive. When it is considered that the FEV₁ values are better in this group than in the whole material, there is no difference regarding prognosis of survival.

The clinical functional group could be estimated in 179 patients. In the best of 3 groups, group A

Table II Survival of 187 patients followed to the end of the study in April 1971

Follow-up (y)	At risk	Dying	Lost ^a	Cumulative proportion surviving	
				%	S.E.
0-1	187	23	0	88	4
1-2	164	16	0	79	
2-3	148	13	0	71	7
3-4	133	14	0	63	
4-5	119	10	27	57	9
5-6	82	11	31	47	
6-7	40	2	3	44	
7-8	13	0	13	44	

Patients observed for fewer years

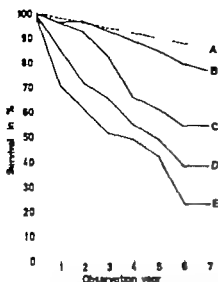


Fig 1 Cumulative survival rate (life table method) of 187 patients according to FEV subgroups. A = survival of "normal population of same age and sex. B = 25 patients with FEV 1.3-1.5 L C = 46 patients with FEV 1.0-1.2 L D = 73 patients with FEV 0.7-0.9 L E = 31 patients with FEV <0.6 L

(patients fit for light work), there was a survival of 76% after 6 years. In group B (patients able to climb staircases but with difficulty or able to walk short distances on level ground but unable to do more than the lightest work) 48% survived after 6 years, and in group C (patients confined to house or bed because of respiratory distress unable to do even light work) the survival after 6 years was 15% (Fig. 2). The relation between clinical functional group and FEV is seen in Table IV and seems to be reasonably good.

The clinical functional group B is quite large and when it is subdivided into two groups according to FEV below and above 0.9 L, there is

Table III Survival of men according to age

	>60 y (n=63)	<60 y (n=70)
FEV <0.9 L	40	35
FEV 1.0-1.5 L	23	35
% dead by end of 6th y	66	46
% expected dead in "normal population of same age after 6 y	29	7.1
% increase in death rate found/expected	2.2	6.8

Table IV Relation between clinical functional groups A, B and C and FEV groups

FEV (lit/sec)	Functional group			Non-evaluable
	A	B	C	
<0.6	1	15	12	3
0.7-0.9	10	47	17	1
1.0-1.2	14	36	4	4
1.3-1.5	10	15	11	0

a 6 years survival of 35% and 63% respectively (Fig. 3).

Patients with ECG signs of cor pulmonale (Le right ventricular hypertrophy or P waves more than 3 mm. were in an overall worse functional group and had a lower FEV value than the rest of the patients (Table V). The patients with cor pulmonale showed a 1% survival after 6 years whereas 35% of those without cor pulmonale were alive after 6 years (Fig. 4).

If a calculation is made according to FEV values for patients with and without cor pulmonale it is found that among the cor pulmonale patients there should have been 51% deaths in 5 years but 65% occurred and among patients without cor pulmonale one would have expected 43% but only 36% occurred. These differences are not statistically significant within the 0.05 confidence level, but highly suggestive.

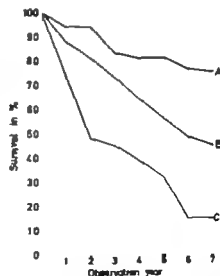


Fig 2 Cumulative survival rate (life table method) of 179 patients according to clinical functional groups. A 35, B 113 and C 31 patients.

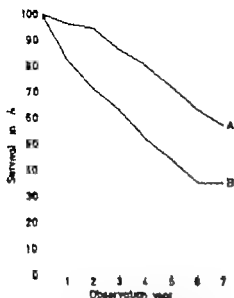


Fig. 3 Cumulative survival rate among patients in clinical functional group B with FEV₁ above and below 0.91 L. A=FEV₁ 1.0-1.5 L. B=FEV₁ ≤ 0.91 L.

In order to facilitate a comparison with other materials the 3 years cumulative death rate for all groups, the S.D. and the 0.05 confidence level are presented in Table VI.

DISCUSSION

All patients investigated include more women than most other similar materials. There is no definite explanation for this, but probably it only reflects different methods of selection and it must be realized that all such materials are highly selected. It is unlikely that chronic obstructive pulmonary disease is more common among women in Denmark than in other countries. In prevalence studies among an urban Swedish population (12, 15) it was found that the relation between men and women with chronic bronchitis was 2.7/1.5-2.1/1.8 depending on the age groups studied. The prognosis of survival after 3 and 5 years according to FEV₁ is very close to the survival found in the studies by Burrows and Earle (5, 6) and Renzetti et al. (22). These authors examined patients of comparable ages and with the same FEV₁ values.

The clinical functional group has a quite good correlation to the FEV₁ demonstrated and the prognosis based on functional group alone shows a significant difference between the groups after 3

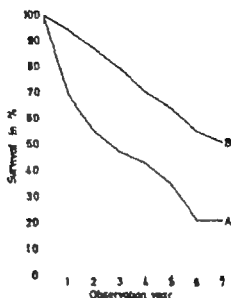


Fig. 4 Cumulative survival rate among patients with (A) and without cor pulmonale (B).

as well as after 6 years of observation. If the functional group and FEV₁ are combined there is an increase in selectivity regarding groups with a better and a worse prognosis. The results for the smaller groups A and C have not been shown in combination with FEV₁ because the S.D. with so small a number of patients will not allow evaluation with any degree of significance. But the tendency is the same and it seems that clinical functional group adds information regarding prognosis to the information obtained through FEV₁ alone.

The results for patients with earlier pulmonary tuberculosis showed no significant difference from results among patients without earlier known tuberculosis but with a similar degree of pulmonary obstruction. These results are in good accordance with the report of Martin et al. (17) who in 611 patients with earlier tuberculosis found mortality to be related to the degree of airway obstruction. They also found that when the tuberculosis was treated the prognosis was related to the remaining degree of pulmonary obstruction.

Age has a significant influence on survival. Men above 60 years had a lethality of 66% after 6 years of observation, while men below 60 years had a mortality of 48%. This difference might, however, be due to the higher number of men with a severe airway obstruction in the older group than in the younger. But the relative increase in

Table V Patients with ECG signs of cor pulmonale in relation to age FEV₁ and clinical functional groups

	Cor pulmonale	
	Positive	Negative
Age (y.)		
0-29		
30-39	1	0
40-49	1	7
50-59	9	4
60-69	20	10
70-	16	31
	1	7
FEV ₁ (l)		
<0.6	13	18
0.7-0.9	77	49
1.0-1.2	6	49
1.3-1.5		23
Functional group		
A	6	79
B	79	84
C	13	18
Non-evaluable	11	8

mortality compared to a "normal" population showed that mortality was 6.8 times the expected in the younger group and .2 times the expected in the older group (Table III). This result, in spite of the generally better lung function in the younger men shows that when the pulmonary obstruction is present in young age the relative increase in mortality is higher than when it occurs at a higher age.

Leiner et al (16) reported that age had a direct relationship to death rate. A decreased maximal breathing capacity in their groups above and below 60 years increased the death rate but the relative mortality in their groups is not evaluated. Simpson (24) reports only the higher risk among younger patients, but it is not possible to see whether the groups are otherwise comparable. Medves and Oswald (18) showed a relatively increased mortality among younger patients than among older in spite of a higher degree of airway obstruction compared to older patients.

When ECG signs of cor pulmonale were present, the survival was considerably reduced even when higher degree of severe airway obstruction in the patients with cor pulmonale was considered. The relatively small number of patients with cor pulmonale does not allow comparison within FEV₁ groups.

Table VI Patients dead after 3 years

N	Category	Dead (%)	SD	x.S.D
187	All patients	79	3.3	22-36
	FEV			
31	<0.6	49	9.0	11-67
75	-0.7-0.9	35	5.5	4-46
56	-1.0-1.1	18	5.2	8-28
25	-1.3-1.5	8	5.4	0-19
	Functional group			
31	C	55	11.0	37-73
113	B	27	4.2	19-35
35	A	17	6.4	4-30
62	B+FEV <0.9	37	6.1	25-49
51	B+FEV 1.0-1.5	14	4.8	4-4
63	Men >60 y	42	6	30-54
70	Men <60 y	25	5.1	15-35
48	Cor pulmonale	53	7	39-67
139	Without cor pulm.	21	3.5	14-28

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THE INFLUENCE OF PORTOSYSTEMIC SHUNT OPERATION ON IMMUNOGLOBULINS AND ESCHERICHIA COLI ANTIBODIES IN PATIENTS WITH CIRRHOSIS OF THE LIVER

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Abstract Serum γ -globulin levels have been determined by paper electrophoresis in 54 patients with cirrhosis of the liver before and after establishment of portocaval shunt. As control group 43 patients operated on for cancer of the lung were studied in the same way. A significant average increase from 18.6 to 23.0 g/l in the cirrhotics was found, whereas no increase in the control group was observed. Serum immunoglobulins and antibodies to *E. coli* O group antigens were studied in 31 patients with cirrhosis and a portocaval shunt and 128 patients with cirrhosis without portocaval shunt. Significantly higher levels of IgG and *E. coli* antibodies were found in the patients with a portocaval shunt. It is proposed that these observations can be explained by the change in liver circulation brought about by the operation. If the cirrhotic liver has retained some of the ability of the normal liver to inhibit immunogens as previously proposed by us, the observed changes in immunoglobulins and *E. coli* antibodies should be expected.

showing a rise in γ -globulins in patients following a portocaval shunt has been published (10) and we have found that antibodies to *E. coli* are more numerous in cirrhotics with a portocaval shunt than in cirrhotics without shunt (7). Experimental portocaval shunt in the rat is followed by an increase in serum γ -globulin (4, 14, 16) and also in antibodies to rat *E. coli* lipopolysaccharide (16).

In order to extend the observations in humans the following two studies were undertaken. 1) A retrospective study of γ -globulin levels in 54 cirrhotics before and after establishment of a portocaval shunt. 2) A study of immunoglobulin levels and of *E. coli* O group antibodies in 31 cirrhotics with a portocaval shunt and in 128 cirrhotics without shunt.

Serum antibodies to common intestinal microbes exist in larger quantities in cirrhotics than in patients not suffering from liver disease (7, 20). This has been explained by the failure of a diseased liver to inhibit immunogens absorbed from the gut thereby causing an increased antibody production against these immunogens. This failure could be caused by abnormalities in the liver circulation (5, 6, 7) or by abnormalities in the Kupffer cells of the liver sinusoids (5, 7, 20). The hypothesis is supported by experimental observations demonstrating an ability of the normal liver to inhibit immunogens (2, 8, 9, 11, 13). If the hypothesis is correct, it should be possible to demonstrate a rise in γ -globulins in cirrhotics in whom a portocaval shunt has been established. One study

PATIENTS AND METHODS (STUDY 1)

During the period 1957-71 169 operations were performed at two municipal hospitals in Copenhagen, establishing portosystemic shunt in patients with cirrhosis of the liver. The diagnosis was proven by means of pre- or peroperative biopsy. In all cases the indication for the operation was haemorrhage from oesophageal varices.

Fifty-four patients (27 men, 27 women) with mean age of 56 years (range 34-82) are selected from those operated on according to the following criteria. 1) Post-operative survival exceeding 1 week without recurrence of haemorrhage. 2) No registered infectious complications during the pre- (4 weeks) or postoperative (12 weeks) periods. 3) Serum γ -globulin concentration determined at least once during the pre- (4 weeks) and postoperative (12 weeks) periods respectively. 4) Pre-operative serum γ -globulin concentration determined

Table 1 Serum albumin and γ -globulin in patients with cirrhosis of the liver before and after portosystemic shunt operation and in patients before and after thoracotomy for pulmonary cancer

	No. of pts.	Albumin (g/l)		γ -globulin (g/l)	
		Mean	S D	Mean	S D
<i>Cirrhotics</i>					
Preop.	54	34.4	6.0	18.6	7.0
Postop. (weeks)					
1-3	46	32.3	4.9	20.2	4.9
7-9	35	32.3	5.8	23.0	8.1
<i>Controls</i>					
Preop.	23	38.3	3.6	12.6	4.9
Postop. (weeks)					
1-3	23	34.6	4.1	13.6	2.5
7-9	23	40.1	4	13.3	2.4

prior to blood and plasma transfusions. The types of portosystemic shunt were side-to-side portocaval (33 cases), end-to-side portocaval (19 cases), and spleno-renal (2 cases).

As a control group 23 patients were selected from the Department of Thoracic Surgery operated on for cancer of the lung. These controls were chosen because the operation in these cases was without direct influence on the portal circulation. The controls were consecutive, but cases with postoperative infections were excluded. In all patients the amounts of blood and plasma

during the pre- and postoperative period registered. The serum albumin and serum γ -globulin concentrations were determined by paper electrophoresis before and after the operation.

RESULTS (STUDY 1)

In Table I pre- and postoperative serum albumin and γ -globulin concentrations are indicated for the 54 cirrhotics and the 23 controls. The postoperative values are divided into two groups according to the time when the analyses were performed but all patients have been studied at least once postoperatively. In the cirrhotics the decrease in albumin concentration is significant ($p < 0.05$ at 1-3 weeks $p = 0.05$ at 7-9 weeks t -test). The rise in γ -globulin concentration is highly significant at 7-9 weeks postoperatively ($p < 0.005$) whereas the rise observed at 1-3 weeks is not significant.

When the 28 cirrhotics in whom the analyses were performed twice postoperatively are studied no significant change in albumin concentration is found and the rise in γ -globulin concentration is

significant both at 1-3 weeks ($p < 0.05$) and at 7-9 weeks ($p < 0.0025$). In the controls a transient decrease in albumin concentration is observed at 1-3 weeks ($p < 0.005$) whereas no significant change in γ -globulin has been found.

Transfusions given to the 54 patients in the pre- and postoperative periods were calculated as plasma 1.3 l (median) varying from 3.0 to 24.2 l. The control patients received 1.1 l (median) varying from 0 to 3.0 l. It is evident that the cirrhotics received much larger amounts of blood and plasma than the controls with a great variation in the quantities. No significant difference between the average γ -globulin increments in cirrhotics receiving less than 7.4 l plasma (25% percentile) and those receiving 15.6 l (75% percentile) or more can be demonstrated. No significant correlation between the amount of plasma transfused and the γ -globulin rises is found in these patients. The average rise in γ -globulin concentration in patients having end-to-side portocaval shunts is equal to that observed after side-to-side portocaval shunts.

PATIENTS AND METHODS (STUDY 2)

In this study 31 patients with cirrhosis and a portocaval shunt and 128 cirrhotics without shunt were investigated. The diagnosis was established in all patients by liver biopsy. Alcoholic and non-alcoholic cirrhosis were equally represented in the groups with and without shunt. Immunoglobulin levels were determined by electrophoresis in antibody-containing agarose gel (Laurell's "rocket" method (15)) and *E. coli* O group antibodies by agglutination titration as described elsewhere (7). Each serum was tested with 10 common O group antigens. A titre of 1/40 or higher was considered positive. The result of the *E. coli* antibody determination is indicated as the number of positive reactions per serum. Some of the results have been published in a study of *E. coli* antibodies in alcoholic and non-alcoholic liver diseases (18).

RESULTS (STUDY 2)

Table II shows the results of the coli antibody and immunoglobulin studies. The average IgG and IgA values are higher in the patients with a portocaval shunt than in those without. The difference is significant with regard to the IgG values ($p < 0.0025$ t -test) whereas the difference between the IgA values is not significant ($0.10 < p < 0.15$). The average number of positive *E. coli* O group antibodies per serum is higher in the patients with a portocaval shunt than in those without. The dif-

Table 11. Antibodies to *E. coli* O antigens and IgG IgA and IgM (g/l) in sera of patients with cirrhosis of the liver with and without portocaval shunt

	No. of pts	No. of pos. antibody reactions		IgG		IgA		IgM	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
With shunt	31	4.6	3.0	24.1	6.8	5.1	2.5	1.41	0.60
Without shunt	128	3.3	2.9	19.0	8.4	4.6	3.3	1.45	1.48

ference is statistically significant ($p < 0.025$ Wilcoxon rank sum test). The intensity of the antibody reactions is the same in the two groups of patients. After exclusion of all prednisone-treated patients (35 patients in the group of cirrhotics without portocaval shunt) the difference with regard to IgG levels and coli antibodies remain significant. There is no difference in the Ig values or number of positive coli reactions per serum between alcoholic and non-alcoholic patients with a portocaval shunt.

DISCUSSION

In the first study the figures shown in Table I demonstrate that a small decrease in albumin concentration and a rise in γ -globulin concentration follow the establishment of a portocaval shunt. A transient decrease in albumin concentration occurred in the control patients operated on for cancer of the lung. The decrease in albumin concentration is similar to alterations found in surgical trauma (17, 21, 22) and is caused by a temporary lateral loss of albumin into the wound oedema and by a temporarily decreased rate of synthesis (17). We believe that the rise in γ -globulin is due to the alteration in the liver circulation brought about by the portocaval shunt. However several important sources of error have to be considered before this result can be accepted. It is not likely that the rise in γ -globulin concentration is an unspecific response to the operation trauma, since there is no rise in the control group and reports in the literature state that there is no increase in γ -globulin in relation to cholecystectomy (22) and gastrectomy (21). Most important as a source of error are the large amounts of blood and plasma given, larger than those given to the controls. Theoretically the plasma infused could cause an increase in γ -globulin for two reasons: retention

of γ -globulin in the vascular space because of its large molecule and retention because of its relatively slow catabolic rate. However the increase in γ -globulin is not related to the amount of plasma given. This would be expected if retention of transfused γ -globulin was the cause of the increase observed.

Furthermore it has been demonstrated in patients with anaemia that transfusions in amounts corresponding to 35% of the blood volume do not change serum γ -globulin concentrations (1). These observations together with our own observations in the controls, also speak against the possibility that antigens in the blood transfusions are the cause of the γ -globulin increase observed. Finally changes in blood volume as possible explanation are not likely as the albumin in our patients is either unchanged or decreasing transiently.

We therefore conclude from this part of our study that the γ -globulin increase observed is related to the change in the liver circulation established by the portocaval shunt. The γ -globulin level was already high before the operation, but the portocaval shunt itself brought about a further increase of the level.

The second part of our study demonstrates that patients with cirrhosis and a portocaval shunt have higher IgG levels and more *E. coli* O group antibodies than cirrhotics without shunt and thus supports the first part of the study.

As far as we know there is only one publication in the literature concerning this problem. Esser (10) has demonstrated an average increase in serum γ -globulin in 107 patients from 1.83 g/100 ml before operation to 2.24 g/100 ml 4 weeks after operation, an increase of the same order as in our material.

The effect of the portocaval shunt on the level of coli antibodies and immunoglobulins can prob-

ably be explained in two different ways. With regard to antigens absorbed directly into the portal blood the fact that the portal blood bypasses the liver in the first circulation may well be of importance for the antibody production. These antigens reach antibody-producing tissues before they reach the greatest part of the RES of the body the Kupffer cells in the liver which probably act as inhibitors of the immunogenicity of the antigens (11-13). With regard to other antigens absorbed into the intestinal lymphatics and the thoracic duct and therefore reaching the liver via the general circulation the explanation cannot be so simple (6). The intestinal microbes absorbed probably belong to this category (1). Here we must presume that the low liver blood flow in patients with a portocaval shunt is the important factor (3-19). The function of the RES of the liver must be much depressed because of the low blood flow and this is probably the explanation of the high level of the colit antibodies.

The high immunoglobulin values may be explained as the sum of these two effects: the bypassing of the liver and the low liver blood flow. The high level of immunoglobulins before the operation is probably due to the fact that these patients already have a pronounced collateral circulation. The increase in the level of the immunoglobulins may reflect the fact that the shunt becomes now even greater and the liver blood flow after.

The fact that alcoholism does not influence the amount of antibodies may be due to the overwhelming influence of the change in circulation so that the smaller effect of alcohol cannot be observed under these circumstances.

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EFFECT OF ORPHENADRINE ON TOTAL THYROXINE

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Abstract. Three clinically euthyroid patients with parkinsonism were found to have raised levels of protein-bound thyroxine ($T_4(D)$). It was demonstrated that treatment with orphenadrine (Disipal®) caused an elevation of $T_4(D)$ of more than 100%. The increase was dose-dependent and the underlying mechanism remained unknown.

The assay of total thyroxine ($T_4(D)$) is based on the specific binding properties of thyroxine-binding globulin (TBG) (5). In contrast to protein-bound iodine (PBI) the determination of $T_4(D)$ is not influenced by the administration of iodine or mercury. However, it has been shown that some drugs can induce pathological levels of $T_4(D)$ in euthyroid patients. Clofibrate and estrogenous compounds increase the binding capacity of TBG which results in elevation of $T_4(D)$ (1-5). Di-phenylhydantoin decreases $T_4(D)$ by competing for the binding sites of TBG (4).

In three patients with parkinsonism we have observed that treatment with orphenadrine hydrochloride (Disipal®) can increase the serum levels of $T_4(D)$.

The patients were clinically euthyroid and had normal values of $T_4(D)$, determined according to Murphy and Jochen (6), and of RT_4U (uptake of ^{125}I -T on protein-coated charcoal) measured as described by Herbert et al. (7). During treatment the $T_4(D)$ level increased in all three patients and this increase seemed to be dose-related (Fig. 1). The RT_4U values remained unchanged. After withdrawal of Disipal the $T_4(D)$ values returned to normal. TSH was normal when measured with and without medication in two of the patients. These two patients (nos. 1 and 3) had also normal urinary excretion of estrone and estradiol during treatment. Elevated TBG values, measured according to Nielsen et al. (7), were found in patient 1 during treatment (222 and 146% normal level 100%) and a slight increase of TBG (117-131%) was noticed in patient 3 during treatment.

The addition of orphenadrine citrate to plasma from a healthy volunteer in concentrations of 1, 5, 10, 50 and 100 µg/ml plasma did not increase the $T_4(D)$ level.

DISCUSSION

It appears likely that the elevation of $T_4(D)$ in these three patients was caused by the treatment with Disipal®. They had normal $T_4(D)$ levels initially but raised levels during treatment with Disipal. Furthermore, normalization of $T_4(D)$ was seen after withdrawal of the medication. The $T_4(D)$ elevation was dose-related in all patients (Fig. 1).

It appears that Disipal does not influence the $T_4(D)$ assay since the $T_4(D)$ was unchanged in all samples after addition of 1-100 µg orphenadrine. The plasma levels of orphenadrine in pa-

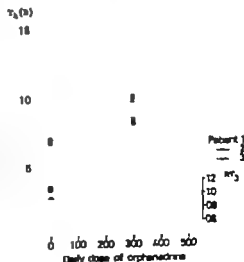


Fig. 1. $T_4(D)$ and RT_4U in three patients treated with orphenadrine.

patients treated with this compound are not known but it is unlikely that they exceed 100 μg or phenadrine/ml plasma at the dosages used.

The increased TBG levels in patient 1 might explain part of the $T_4(D)$ elevation but this is not in agreement with the minor increase of TBG in patient 3. Another possibility of the effect of orphenadrine on $T_4(D)$ would be that the compound increases the binding capacity of TBG. However, the RT_3U levels did not decrease. Thus the mechanism remains obscure.

Hansen and Sierbaek-Nielsen (2) studied the PBI levels in 50 patients treated with perphenazine. Nine of their patients had PBI levels higher than 8 $\mu\text{g}/100\text{ ml}$. It is remarkable that five of these nine patients were also on orphenadrine treatment. It is not unlikely that the PBI elevation in these patients was caused by orphenadrine.

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PRIMARY AMYLOIDOSIS WITH POLYNEUROPATHY

Some Aspects on the Histopathological Diagnosis Ante Mortem based on Studies of Biopsy Specimens from 30 Familial and Non-familial Cases

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Abstract. Biopsy specimens from 30 patients with primary amyloidosis and polyneuropathy have been reviewed with regard to the histopathological methods used and to the kind of tissues examined. The importance of using modern histopathological methods to establish the amyloid diagnosis was demonstrated. Biopsy of the rectal mucosa of 18 patients was positive for amyloid in all. Skin biopsy was positive in 22 of 25 patients. In one patient the first skin and rectal biopsies were negative; new specimens after one year and three years, respectively, were positive for amyloid. In all 30 patients amyloid deposits were found either in the rectal mucosa or in the skin. Rectal and skin biopsies are recommended as valuable procedures to confirm the clinical diagnosis of primary amyloidosis with polyneuropathy.

During the last few years about 40 cases of primary amyloidosis with polyneuropathy most of them familial have been diagnosed in the north of Sweden. In the first cases some difficulties were encountered in proving histologically the occurrence of amyloid deposits (?). As a conclusive diagnosis of amyloidosis depends on histopathological verification, we have found it valuable to review the results of our examinations with regard to the kind of tissues investigated and to the methods used. The main purposes of this study were to see whether skin biopsy was as valuable among our patients as it is reported to be in Portugal (14), to review our results of rectal biopsy and to compare the results of the skin and rectal biopsy procedures. The present report will also serve to illustrate the importance of using modern sensitive histopathological methods (11-13) to

demonstrate the amyloid deposits also in this type of amyloidosis.

MATERIAL

The material consisted of 30 patients: 1 men and 9 women. Three patients were found during a pathological investigation of the disease. The others were admitted to hospital because of more or less distinct symptoms and signs of polyneuropathy. Other clinical manifestations were gastrointestinal and urinary bladder disturbances, heart affection and renal disturbances because of chronic nephropathy in 21 patients, 13 men and 8 women; the disease was found to be familial. The families are indicated by capital letters, from A to G. To each familial case is added a number. Nine patients considered sporadic so far are indicated by capital letters only from H to P. Reports concerning some of these cases have appeared previously (1, 3, 4, 9). The histological confirmation of amyloid deposits was made ante mortem in 76 cases. In three cases (A.5, A.7, B.2) it was not made until after the patient's death at reexamination of biopsy specimens taken before death. In one case (H) the diagnosis was proved first at autopsy. In no case was chronic inflammatory disease, myelomatosis or tumour detected.

Data concerning sex, age at biopsy and duration from onset of clinical symptoms are given in Table 1. The clinical evaluation of the neuropathy which appeared first and most markedly in the legs, also shown in Table 1. It is graded semiquantitatively as in preceding report (3). In 15 patients the neuropathy in the legs was marked (+++) moderate (++) in 11 and slight (+) in 4. Clinically no significant differences were found when repeated examinations were performed.

METHODS

In all patients biopsy was performed ante mortem for histological examination. Skin specimens were taken at

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Table 1 Examination of amyloid deposits in biopsy specimens from 30 cases of primary amyloidosis with polyneuropathy

Case	Sex	Age at biopsy (y)	Duration of symptoms (y)	Clinical grading of neuropathy in legs ^a	Tissue specimen	Examination of amyloid deposits	
						Methyl violet (Positive)	van Gieson's stain (Negative)
A.1	♂	66	16	+++	Nerve skin rectum, muscle liver kidney tongue gingiva	Nerve	Skin, rectum, muscle liver kidney tongue, gingiva
A.2	♀	63	10	+++	Nerve skin, muscle	Nerve	Skin, muscle
A.3	♂	70	24	++	Muscle urinary bladder		Muscle urinary bladder
A.6	♂	61-64	3-6	+++	Rectum muscle (61) skin (64)		Rectum muscle ^b (61)
A.7	♂	68	18	+++	Skin rectum muscle liver gingiva		Skin, muscle, rectum, liver gingiva
B.1	♀	61-61	4-5	+++	Nerve rectum (60), skin rectum (61)	Nerve	Rectum (60)
B.2	♀	68-70	5-7	+++	Nerve skin, rectum muscle (68), salivary gland (70) ^c	Salivary gland (70) ^b	Nerve, skin, rectum, muscle (68)
B.3	♂	43	3	++	Skin, rectum		
B.6	♂	57	6	++	Nerve skin jejunum		
B.7	♀	35	2	+	Skin		
B.8	♀	40-43	1-3	+	Rectum (40), skin (42), skin rectum (43)		
B.9	♂	63	3	++	Skin		
B.10	♀	35	2	+	Skin		
C.2	♂	32-34	3-5	+++	Skin muscle jejunum (32) skin (34), nerve skin rectum (34)		Skin, ^b muscle jejunum ^b (32)
D.1	♂	66	3	+++	Rectum		
D.2	♂	68	5	+++	Rectum		
D.3	♀	60	13	+++	Rectum		
E.1	♀	63	9	++	Skin rectum muscle		
E.2	♂	70	14	+++	Nerve skin		
F.1	♂	62	6	++	Skin rectum		
G.1	♂	57-59	1-3	++	Skin (57) nerve rectum (58) skin (59)		Skin ^b (57)
	♂	51	2	+++	Liver jejunum		Liver jejunum ^b
	♀	64-66	7-9	++	Skin rectum (64) rectum (65) rectum (66) skin rectum (66)		Rectum (64)
	♂	78	2	++	Skin, rectum		
K	♂	54	4	++	Skin		
L	♂	57	7	+++	Skin		
M	♂	69	4	+++	Nerve skin skin		
N	♂	61	15	++	Skin		
O	♂	73-5	3-5	+	Rectum (73) skin (75)		
P	♂	78	10	+++	Skin rectum		

+ = slight, ++ = moderate, +++ = marked

Amyloidosis clinically not suspected at the time of examination

Figures within parentheses = patient's age at the time of repeated biopsy

small punch biopsies. The inner diameter of the rotating cylinder knife used was 2 mm. In general we have performed the skin biopsies in the distal part of the lower leg. We have tried to perform the biopsy adjacent to hair follicles in order to get arrector pili muscles in the specimens. Biopsy of the rectal mucosal membrane was made with a pair of conventional biopsy forceps. Surgical biopsies were made of the sural nerve and of striated muscles of the lower leg. Tissue specimens were fixed in 10% formalin and routinely embedded in paraffin. Two examination procedures were used:

(I) Before the alkaline Congo red method had been introduced in our laboratory sections routinely stained

with van Gieson's stain or with hematoxylin and eosin were examined for the presence of deposits that could represent amyloid. If such deposits were present staining with methyl violet was also performed. All specimens were later reexamined according to procedure II.

(II) After the alkaline Congo red method (13) had been introduced as a routine method in our laboratory all biopsy specimens from cases supposed to suffer from amyloidosis were investigated according to that method. Sections stained by the alkaline Congo red method were examined light-microscopically both in ordinary and in polarized light (11).

Alkaline Congo red and polarized light (Positive)	(Negative)
Nerve, skin, rectum, muscle, tongue, gingiva	Liver kidney
Nerve, skin, muscle Urinary bladder Rectum, muscle (61), skin (64) Skin, rectum, muscle	Muscle Liver
Nerve, rectum (60) skin, rectum (61) Nerve, skin, rectum, muscle (68), salivary gland (70)	
Skin, rectum Nerve, skin Skin Skin, rectum (43)	Jejunum Rectum (40) skin (42)
Skin Skin Skin, muscle, jejunum (32) skin (34), nerve, skin, rectum (34)	
Rectum Rectum Rectum Rectum Nerve, skin Rectum Skin (57), nerve, rectum (58) skin (59)	Skin, muscle Skin Liver Jejunum
Skin, rectum (64), rectum (65), rectum (66), skin, rectum (66) Skin, rectum Skin Skin Nerve, skin, skin Skin Rectum (73) Skin, rectum	Skin (75)

RESULTS

The various tissue specimens taken for microscopic examinations and the results are shown in Table I. Especially in some of our first patients (A.1, A.7, B.2, C.2) several biopsies were performed.

(I) By method I deposits of an amorphous picrophilic substance were found in the sural nerve

of cases A.1, A.7, and B.1 and in sections from a submandibular salivary gland of case B.2. The substance showed a metachromatic reaction with methyl violet. Otherwise examinations with these staining methods were negative for amyloid in many specimens (Table I).

(II) Light-microscopical examination, both in ordinary and polarized light after staining with alkaline Congo red, revealed amyloid deposits in most of the specimens (Table I). Of 82 specimens examined in that way amyloid was detected in 69. No amyloid was found in 4 biopsies of the skin (E.1, F.1, 0 and the first skin biopsy of B.8). No amyloid was found in 3 biopsies of the liver (A.1, A.7, H). 2 of striated muscles (A.5, E.1) of the jejunum (B.6, H). 1 of the kidney (A.1) or in the first rectal biopsy of patient B.8.

The clinician assumed a diagnosis of amyloidosis in 8 patients from whom 23 biopsy specimens were examined by both methods. With methyl violet and van Gieson's stain amyloid deposits were detected in 3 specimens from peripheral nerves only (*vide supra*). On examination with polarized light after staining with alkaline Congo red, however, amyloid was found to be present in 19 of the 23 specimens (Table I). No amyloid was detected in biopsy specimens from the liver (A.1, A.7, H) or from the kidney (A.1).

Amyloidosis was clinically not assumed at the time when the examination of 10 biopsy specimens from 7 patients was performed (Table I). By method I amyloid was found in the salivary gland of case B.2 only. (This biopsy was performed because of a tumourlike enlargement of a submandibular salivary gland.) Subsequent re-examination by method II revealed amyloid deposits in 7 additional specimens of 5 patients (Table I).

Biopsy was performed of the rectal mucosal membrane of 18 patients. Amyloid was found in all of them. Amyloid was detected in altogether 22 of 23 rectal biopsies. The deposit of amyloid was found to be located particularly in the muscular layer of the mucosa (Fig. 1). In specimens containing parts of the submucosa amyloid appeared in vessel walls and in the perivascular connective tissue.

Thirty-one specimens of skin biopsy from 25 patients were examined by polarization microscopy after staining with alkaline Congo red. Amyloid deposits were detected in 27 specimens from 22 cases. It was found in 3 of 4 patients with slight



Fig 1 Section from the rectal mucosa of case B-1. In partly polarized light each area containing amyloid appears as bright green (white in the photomicrograph) and red (black) patches. Most of the amyloid in the muscular coat of the mucosa (m.m.) is found in its deeper parts. Two arrow heads indicate the largest deposit located there. Small deposits (arrows) can also be seen in the upper parts, close to the crypts. Alkaline Congo red stain and partly polarized light. $\times 135$

and in 8 of 10 with moderate manifestations of neuropathy in the legs at the time of examination. In 11 patients with marked signs of peripheral neuropathy amyloid was found in the skin of all

The amyloid deposit was often found to be adjacent to the arrector pili muscles (Fig.

An relation to the sweat glands and in vessel walls

Both skin and rectal biopsies were performed on 20 occasions in 15 patients. Amyloid was found in 19 of the 20 rectal and in 16 of the 20 skin biopsy specimens. The patient with both skin and rectal biopsies negative for amyloid at the first examinations (B.8) had only a slight degree of peripheral neuropathy.

We performed biopsy of the *sural nerve* in 9 patients. Amyloid was detected in all specimens (Table I). Seven of these patients had marked and two moderate peripheral neuropathy. In the nerves the amyloid deposits were located especially in the endo- and perineurium often appearing as rounded masses (Fig. 3).

Amyloid was found in biopsy of the *jejunum* of 1 case whereas no amyloid was detected in such specimens of 4 other cases (Table I). In some other patients e.g. A.7, B.7 and J. repeated trials

were made to get the biopsy instrument through the pyloric region but all were unsuccessful.

In 8 patients biopsy was performed of *striated muscles* (Table I). Varying degrees of neurogenic atrophy of the musculature were observed. Deposits of amyloid were found in 6 patients who all had marked clinical signs of peripheral neuropathy. The deposits, however were scanty appearing chiefly in the vessel walls and in the nerves. The two patients without any amyloid in the striated muscle specimens had more moderate manifestations of peripheral neuropathy.

Liver biopsy was examined in 3 cases only. In none of them could any amyloid be detected.

COMMENTS

The preferential reaction of amyloid to crystal violet and methyl violet, termed metachromasia, was found to be a useful staining method for tissue sections (7). So was staining with Congo red (6) which gives amyloid a pink colour. Some times however it may be difficult to demonstrate amyloid with these staining methods. Therefore it was a great step forward when it was observed that amyloid gave a green birefringence after staining with Congo red when viewed in the polarization microscope (11). By staining with alkaline solution of Congo red (13) the examination achieved some advantages. It seems to be a really



Fig 2 Part of biopsy specimen from the skin of case A.2. Amyloid (black) forms the border of arrector pili muscles (p). Alkaline Congo red stain. $\times 129$

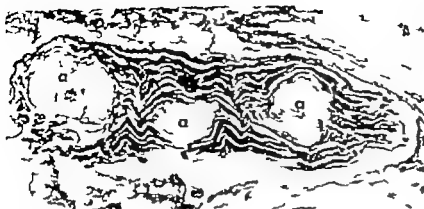


Fig. 1. Section from the sural nerve of case A. Three rounded patches of amyloid (□) are shown. Van Gieson stain. 165

sensitive method for demonstrating even small amounts of amyloid. Our study shows that it is also of importance for the examination of biopsy specimens from patients suffering from amyloidosis with polyneuropathy.

Examination of biopsy specimens from the rectal mucosa often reveals amyloid accumulation in systemic amyloidosis (8). In preceding reports on familial amyloidosis with polyneuropathy, however, the results of rectal biopsies have been described only infrequently (14). Our observations show that biopsy of the rectal mucosa is a valuable procedure to demonstrate amyloid deposit in this type of amyloidosis. In agreement with reports on the distribution of amyloid deposits in the stomach and small intestine (12, 15) we found that the amyloid substance in the rectal mucosa was located especially in relation to the muscular layer. This part of the mucosa thus has to be included in the biopsy specimen in order to get relevant material.

Skin biopsy has been reported to be a useful diagnostic procedure in familial amyloidosis with polyneuropathy. In 24 biopsy specimens of the skin from 23 patients in Portugal amyloid was detected in 23 (14). In our cases, too, examination of skin biopsy specimens was found often to reveal amyloid deposits. In agreement with reports from Portugal (14) we often found amyloid deposits located adjacent to the arrector pili muscles in relation to sweat glands and in vessel walls.

When comparing the results of examinations of rectal and skin biopsies in our material amyloid was found almost as frequently in the skin as in the rectal specimens. As the symptoms and signs

of peripheral neuropathy in our patients appeared first and most markedly in the lower limbs, we think that skin biopsy of the lower part of the leg may be an appropriate procedure. It is easy to perform and if it is negative in cases initially supposed to have amyloid deposits, such a biopsy can easily be repeated. Biopsy of the rectal mucosa is a useful alternative or supplementary procedure.

To judge from our observations it is obvious that biopsy of a peripheral nerve also may be a useful alternative. In cases with marked neuropathy and with disturbances of the sensibility this procedure is harmless. In such cases, however, biopsy of the skin and rectum may be expected to reveal amyloid as well. In patients with only discrete symptoms of neuropathy, nerve biopsy may be more conclusive than biopsy of the skin or rectum. As far as we know, however, this question has not been studied and has to be left open.

In Portugal, where gastrointestinal disturbances also occur frequently among patients with this disease (5), biopsy of the stomach (17) and of the small intestine (15) has been reported to reveal amyloid deposits. Because of the gastric neuropathy with disturbance of motility and delay in emptying of the stomach we found it difficult to pass a biopsy capsule beyond the pyloric region. Therefore, in this study we were able to get biopsy specimens of the jejunum in only a few cases. Biopsy of the gastric mucosa was not performed.

A progressive atrophy of striated muscles, especially in the distal parts of the limbs, is typical of this disease. Amyloid may appear in the walls of

larger blood vessels located interstitially in the musculature. It is apparent from this study however and from our examination of autopsy cases (10) that, even in the presence of advanced muscular atrophy no or only minor amounts of amyloid are to be found in the rather small specimens usually taken at skeletal muscle biopsies.

As we have found at autopsy examinations (10) and as also reported from Portugal (14) amyloid accumulation in the liver is usually scanty in this form of amyloidosis. It appears in the vessel walls of the portal triad only. In none of our 3 liver biopsies could amyloid be detected. The amyloid involvement of solid viscera including the liver however may be variable and more extensive in other families. Thus in a report from Iowa, USA (1) concerning familial cases of amyloidosis with polyneuropathy nephropathy and peptic ulcer the examination of biopsy specimens of internal parenchymatous organs was said to be an appropriate procedure.

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TREATMENT OF ACUTE LEUKEMIA IN ADULTS

Results with a Combination of Potent Cytotoxic Drugs

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Abstract A total of 66 adult patients with acute leukemia were seen in the 2-year period May 1971-May 1973. In 4 patients the rate of progression was judged to be slow and they were therefore left untreated. Two patients with acute myeloid leukemia (AML) died 12 hours and 7 days, respectively after start of treatment and are excluded as non-evaluable. The remaining 60 treated patients include 8 acute lymphatic leukemias with 7 remissions, 6 stem cell leukemias with 5 remissions, and 46 AMLs with 25 remissions. Treatment of 26 patients with AML started more than 12 months ago: 16 of these went into remission and have a median survival of 14 months, compared to 4 months in the 10 not obtaining remission. Five AML patients are in complete remission, 28, 27, 26, 23 and 22 months, respectively after start of therapy.

Until about 5 years ago the results of treatment of patients with acute myeloid leukemia (AML) were very poor and many hematologists therefore only gave supporting treatment to AML patients. During the last 5 years treatment with combinations of daunorubicin, cytosine arabinoside (and sometimes other drugs in addition) have in some centres resulted in remission rates of more than 50% (1, 2, 5).

In May 1971 we started treating adults with acute leukemia with a combination of potent cytotoxic drugs. Our combinations of drugs were only a slight modification of those of Splers (6).

MATERIAL

Our Section of Haematology receives patients referred from hospitals in all parts of Norway. We have accepted for admission any patient with newly discovered acute leukemia between 15 and 60 years of age except for 3 patients who had previous severe coronary artery disease. (These 3 patients were not admitted and were not

given the opportunity of treatment with our combination of potent cytotoxic drugs. However they were not more critically ill than many of those admitted for treatment and might have obtained remission if they had been given the opportunity of the potent treatment.) Three patients above 60 years of age (64, 64, 62 years) have been treated.

All patients have been admitted to our section within 2 days of referral. We have started chemotherapeutic treatment as soon as the patient has been admitted, regardless of fever etc.

Classification of acute leukemia

Our differentiation between acute myeloid (AML) and acute lymphoblastic leukemia (ALL) was mainly based on the appearance of the primitive cells (May) Grönwald-Giemsa-stained blood and bone marrow films: (a) More than 5% promyelocytes indicated acute myeloid. (b) Auer rods were diagnostic of acute myeloid. (c) If the blast had a free rim of cytoplasm larger than 1/3 of the area of the nucleus this suggested acute myeloid. If the free rim was less than 1/4 of the area of the nucleus it suggested ALL. (Unfortunately the free rim was often between 1/4 and 1/3 of the area of the nucleus, leaving one undecided.)

TREATMENT

AML (all variants). We generally treated for 5 consecutive days of each 2-week period during the first year and for 3 consecutive days of each 3-week period during the second year. Our combinations of four drugs and our method of treatment was only slight modification of that of Splers (6). The first six (induction) courses of treatment consisted of daunorubicin 60 mg i.v. on day 1, only cytosine arabinoside 150 mg i.v. daily for 5 days, prednisone 40 mg by mouth daily for 5 days, and either 150 mg 6-mercaptopurine or 120 mg thioguanine by mouth daily for 5 days.

The next ten (maintenance) courses consisted of six which were exactly like the induction courses, and two courses in which 60 mg daunorubicin on day 1 was replaced by 2 mg vincristine i.v. and 150 mg 6-mer-

captorine was replaced by 190 mg cyclophosphamide i.v. daily for 5 days. The last two maintenance courses consisted of vincristine 2 mg i.v. on day 1, methotrexate 7 mg by mouth daily for 5 days, prednisone 40 mg daily for 5 days, and 6-mercaptopurine 500 mg by mouth daily for 5 days.

These ten maintenance courses were then repeated over and over again, two courses containing vincristine always following after three courses containing daunorubicin.

If the bone marrow became blast-free but hypoplastic the free interval between two treatment courses was sometimes increased from 9 to 16 days, even during the first year of treatment.

ALL, age 15-20 years. We gave vincristine 2 mg/m² i.v. once a week for 3 or 4 weeks, prednisone by mouth 40 mg/m²/day for 4 weeks and 6-mercaptopurine 100-150 mg daily. This usually resulted in remission within 3-4 weeks. The remission was then maintained with 6-mercaptopurine 100-150 mg daily. Reinforcement of the remission with vincristine 1 was given every 3 months. When the patient obtained hematological remission CNS prophylaxis was given with 5 intrathecal injections each of 20 mg methotrexate. Subsequently 20 mg methotrexate was given intrathecally twice every 3 months.

ALL, age above 20 years. We used a treatment which was only slightly modified from that of Spiers (6). This regimen resembles that used for AML, but the combinations of four drugs is slightly different. The first six (induction) courses of treatment consisted of vincristine 2 mg i.v. on day 1, only cytosine arabinoside 150 mg i. daily for 5 days, cyclophosphamide 150 mg i. daily for 5 days and prednisone 200 mg daily for 5 days.

The next eight (maintenance) courses consisted of which were exactly like the induction courses and courses consisting of daunorubicin 60 mg on day 1, cytosine arabinoside 150 mg i. daily for 5 days and 6-mercaptopurine 150 mg by mouth daily for 5 days. The two remaining maintenance courses consisted of vincristine 2 mg on day 1, methotrexate 7 mg by mouth daily for 5 days, prednisone 200 mg daily for 5 days and 6-mercaptopurine 500 mg by mouth daily for 5 days.

If the bone marrow became blast-free but hypo-

plastic the free interval between two treatment courses was sometimes increased from 9 to 16 days, even during the first year of treatment. If the patient developed signs of paresis of the fibular muscles (unable to walk on his heels) vincristine was omitted from one course of treatment.

Unclassified stem cell leukemia. These patients were treated like ALL patients above 20 years of age.

Supporting care

In the initial phase we gave liberal amounts of fresh blood transfusions. Sometimes platelet-rich plasma was given for thrombocytopenia with bleeding, but we did not have facilities for granulocyte transfusions.

Severe granulocytopenic patients were kept in single rooms and visitors kept to a minimum, but life stand or laminar air flow technique was not available.

We did not use prophylactic antibiotics or bowel sterilization, but we started immediately with large doses of antibiotics in febrile patients.

Criteria of remission

(a) Less than 5% blasts in the bone marrow and less than 10% promyelocytes. (b) Platelets within the normal range at least in some part of the treatment cycle. (A somewhat reduced number of granulocytes and some degree of anemia may be caused by toxic action on bone marrow or hemolysis, due to the cytotoxic drugs.)

RESULTS

Table I gives a survey of the 66 new cases of acute leukemia admitted during the 2 year period May 1971 - May 1973. Four of the 66 patients were not started on the combination of potent cytotoxic drugs because they were considered to be of the slowly progressing type not likely to benefit from it. Table II groups the treated patients according to whether they obtained remission or not. Two cases have been excluded as not evaluable: one died 12 hours, the other 7 days after admission. Table III lists the median survival for the patients obtaining and for those not ob-

Table I. New cases of acute leukemia in May 1971 - May 1973

Type	No of pts	Age (y)		Treated	Not treated
		Range	Median		
ALL	8	13-33	31	8	
Stem cell	6	26-59	43	6	
AML					
"Classical"	27	15-64	46	26	1
Myelomonocytic	18	17-69	41	18	
Erythroid	6	43-77	61	3	3
Hypergranular promyelocytic	1		38	1	
Total	66			62	4

Table II Treated patients in different variants of ALL grouped according to remission or no remission

	No. of pts.	Remis- sion	No re mission	Age (y)	
				Range	Median
<i>Treated</i>					
ALL	8	7	1	13-30	18
Stem cell	6	5	1	26-59	33
AML (all variants) ^a	46	25	21	16-61	40
				15-69	46
Total	60	37	23	13-62	35
				15-69	43
<i>AML</i>					
"Classical"	26	13	13	16-61	38
				15-64	49
Myelomonocytic	17	10	7	18-58	41
				17-69	41
Erythrocytic		1	1		61
					54
Hypergranular promyelocytic ^b	1	1			38
Total	46	25	21	16-61	40
				15-69	46

^aTwo patients who died are excluded.
^bSurvived (7).

taining remission. Table IV shows median survival for all the treated patients regardless of whether they obtained remission or not.

DISCUSSION

It has been stated (3) that chemotherapy in AML is least likely to benefit patients whose disease processes are progressing at either a very rapid or very slow rate.

Patients acutely and desperately ill when first diagnosed may die before they reach the leukemia centre from the local hospital. Also some centres will initially only give antibiotics and supporting

treatment to desperately ill patients and only those who get over their infection etc. will be put on a chemotherapeutic regimen (3). A variable portion of the poorest risk AML patients may in these ways be omitted from the trials in larger centres like our section. This has to be kept in mind when comparing results from different hospitals. The remission rate among all patients diagnosed as AML in a general city hospital will for these reasons tend to be lower than in larger leukemia centres like ours to which patients are referred from local hospitals.

The slowly progressing patients probably represent a relatively small fraction of the patients

Table III Median survival for patients started on treatment more than 12 months ago grouped according to remission or no remission

	N of pts.	Remis- sion	No re- mission	Median survival (mo)	Age (y)	
					Range	Median
ALL	7	6	1	18	13-30	21
				1		33
Stem cell	5	4	1	17	26-59	41
				2		43
AML (all variants)	26	16	10	14	17-61	38
				4	31-64	46

BOOK REVIEW

Environmental Health Aspect of Lead International symposium Amsterdam October 2-6 1972 1168 pp. Price c. 265 Sw cr CID EG May 1973

In Oct. 1972 a common symposium on plumbism was organized in Amsterdam by the Commission of the European Communities (CEC) and the United States Environmental Protection Agency (EPA)

Among the 300 specialist from all over the world there were physicians, biochemists, toxicologists economists and many other professions. The problems therefore could be treated from many points of view. In the seven sections there were more than 90 papers. Among the subjects treated were the presence of lead in the external milieu, the question how the metal reaches the human being, the incorporation of lead in

the human organism, the toxicology and metabolism of lead in different animal species, cytotoxic and biochemical effects of lead in vitro, subclinical effects of lead and their usefulness for the diagnosis of early lead poisoning, the epidemiology of plumbism and, finally, methods for the analysis of lead. There was also a round table discussion with a panel judging a wide data regarding the detrimental influence on the human organism.

The volume is very well edited and should become a standard work for everybody who is interested in lead problems. Most of the acute problems in the discussion about plumbism are presented in an understandable way. The language is almost entirely English. German and French papers have an English summary.

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MULTIPLE JEJUNAL STRICTURES ASSOCIATED WITH MEGALOBlastic ANAEMIA AND MYELOPATHY

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Abstract In patient with multiple strictures chronic stasis and dilatation of the jejunum. Neurological defects suggestive of subacute combined degeneration of the spinal cord developed over 10-year period. In spite of optimal substitution therapy with vitamin B₁₂ the bone marrow was megaloblastic, which was ascribed to folic acid deficiency resulting from malabsorption of folic acid. A possible association between the neurological lesions and the folic acid deficiency was not established, as there may also have been deficiency of other nutritional factors of significance for nervous tissue. Malabsorption carries a poor prognosis in this condition, but in the case presented all symptoms of malabsorption were relieved by surgical removal of the jejunal strictures.

The association between jejunal strictures and "pernicious anaemia" was first described by Faber (10) in 1897. So far relatively few observations of this "stricture anaemia" have been reported. In 1969 Chanarin (6) traced 51 cases in the literature and an additional case was published in 1971 (2).

The diagnosis is difficult to establish. In many cases the patient is young and has suffered from dyspepsia for several years. Nausea, colicky pain in the epigastrium, abdominal rumbling and projectile vomiting are conspicuous symptoms. Steatorrhea may be a pronounced feature. Weight loss usually occurs, and in 25% of the patients neurological defects develop (3).

Meulengracht (14) observed that liver therapy could bring about remission of the anaemia, and most of the patients in his study seemed to suffer from vitamin B₁₂ deficiency. Folate deficiency may also be present (2). Surgical correction of the anatomical abnormality leads to remission of the megaloblastic anaemia, but owing to the poor general condition of the patients the operative mortality is high (5).

The pathogenesis of this stricture anaemia is unknown. Faber (10) assumed that toxin produced by the stagnant intestinal contents was the cause of the anaemia—a view which was subsequently supported by Thompson and L. Gley (17) who suggested that a toxin produced by intestinal bacteria led to haemolysis which in turn resulted in vitamin B₁₂ and folic acid deficiency. Cameron et al (5) presumed that the intestinal stagnation inhibited the absorption of one or more haemopoietic factors and Halsted et al (12) explained the megaloblastic anaemia by vitamin B₁₂-consuming bacterial flora in the stagnant intestinal contents.

In the case described here malabsorption of folic acid and vitamin B₁₂ were outstanding features which however could be relieved by surgical correction of the anatomical defect.

CASE REPORT

The patient, male, then aged 16 years, was admitted to hospital for the first time in 1960 with complaint of persistent diarrhoea. In childhood he had been in good health. The dyspeptic symptoms had begun about 1 month previously with colicky epigastric pain especially after meals. Occasionally projectile vomiting—without preceding nausea—occurred, resulting in relief of the pain. The attacks were sporadic with free periods of 1-2 months. No weight loss had occurred. During the hospital stay the stools were voluminous and loose but did not appear to be fatty. Stool examinations for blood were negative, and barium meal studies did not reveal any abnormalities.

In view of the indefinite findings no treatment was given and the condition remained largely unchanged until 1966 when the patient was readmitted with diarrhoea and borborygmi.

The stools were now suggestive of steatorrhea, and an examination showed excretion of large amounts of



Fig. 2. Barium meal study (1970) showing multiple strictures and dilatation of the jejunum.

fat in the faeces (>30 g/24 h). A before the patient complained of colicky pain which was relieved by vomiting. His nutritional condition was poor: height 175 cm, body weight 48 kg. Decreased absorption of lactose, glucose and D-xylose and slight anaemia were revealed, but the bone marrow was normal, and so was the vitamin B₁₂ concentration. The FIGLU test (fornixisomoglutaric acid; 5-hou urine specimen after hydrous load) showed increased values, indicating folate deficiency (Fig. 1). The Schilling test with and without intrinsic factor yielded pathologically low values. Serum calcium and serum protein concentrations were depressed. No episodes of tetania were observed. Barium meal studies revealed retention after 12 hours, but no gastric ulcers. The intestinal motility was depressed and the duodenum was found to be dilated.

Malabsorption was thus present, and even though the clinical picture was not quite characteristic of coeliac disease (Fig. 1) together with substitution therapy with injections of vitamins B₁₂, A and B in complex preparation. For some time the patient was also given oral folic acid, 5 mg three times daily.

After discharge the patient was seen in our Out patient Clinic every fourth week. There was no definite effect of the dietetic treatment, and as the patient described some of his abdominal discomforts to it, the dietary regimen was abandoned. He began to complain of paresthesias of both lower limbs, and owing to increasing fatigue he was then readmitted in 1969 for further malabsorption studies which revealed severe steatorrhea and pathological values of the Schilling, xylose and FIGLU tests. Free acid was present in the stomach. There was megaloblastic dysplasia in the granulopoietic (poor metamyelocytes, giant stab cells

and macropolocytes characteristic of maturation disturbances) deficiency of antimegaloblastic factors (4)) and the serum folate concentration was low. A the folic acid absorption (TRIFA) test (9) yielded low pathological values the administration of folic acid resumed X-rays of the stomach and small intestine showed increasing dilatation of the duodenum and the upper portion of the jejunum, but the jejunal motility was apparently normal.

After discharge the patient became increasingly depressed by his condition and failed to return for check-ups for nearly 1 month, during which time he did not receive any substitution therapy. On account of increasing numbness and aysaergia of the legs he returned in 1970 and was again admitted for renewed studies. He was now greatly emaciated and in poor general health. Neurological examination revealed pronounced ataxia, decreased deep and surface sensibility and spastic paresis of both lower limbs. He could walk with two forearm crutches, but was absolutely helpless in the dark.

The biochemical status was largely unchanged from the previous admission. Gastrocamera examination was technically successful (1) showing normal stomach. As before, X-rays of the small intestine showed dilatation of the duodenum and the upper portion of the jejunum. In addition, several exposures (Fig. 2) aroused suspicion of stenoses of the jejunum about 1 m distal to the Treitz ligament. Fibroscopy revealed fluid surfaces in the dilated jejunal loops, but the peristalsis preserved.

Owing to the continued progression of the patient disease—in particular the neurological defects—it was decided to perform exploratory laparotomy with view to relieving mechanical obstruction if any. The operation revealed an intestinal tessel (treated about 74 cm distal to the Treitz ligament, followed by dilated segment and then by another stenosis (Fig. 3). Proximal to the stenosed segments the jejunum and duodenum were markedly dilated. The intestinal wall as hypertrophied, and the segments showed vigorous peristalsis. The stenosed portions of the intestine (35 cm in length, were resected, and an end-to-end anastomosis between the normal-appearing jejunum and the dilated proximal intestinal segment was carried out. The postoperative course was uneventful. Histological examination of the resected specimen showed acuteres of an unspecific inflammation of the stenosed portions while the rest of the intestinal tissue was normal, apart from hypertrophy of the lamina muscularis.

The substitution therapy was continued unchanged, and 6 months after the operation the patient was readmitted for malabsorption studies. The Hb content was 151 g/100 ml with 4.34×10^9 erythrocytes/ μ l, the peripheral blood and bone marrow were normal. The Schilling test with and without intrinsic factor the FIGLU TRIFA and xylose test showed normal values. A moderate steatorrhea of 7 g/24 hours persisted. X-rays of the small intestine showed less dilatation than on the previous occasions; the intestinal motility was normal.

Some neurological defects still

but they



Fig. 3 The strictureed jejunum with dilated proximal segment.

seemed to be slowly regressing. One year after the operation the patient's general wellbeing was improved so much that he could be restored to his work as a photographer.

DISCUSSION

The patient described suffered from malabsorption, megaloblastic anaemia and neurological defects gradually resulting in severe disability. He had for a long time had dyspeptic discomfort which could be ascribed to the two strictures of the upper jejunum. Apart from the irreparable neurological lesions, all symptoms as well as the biochemical and physiological disturbances were relieved on reestablishing the intestinal passage by removal of the stenosed segment. The malabsorption syndrome may therefore be assumed to be a consequence of the intestinal stenosis and dilatation.

It is not possible to offer an aetiological explanation of the stenosing process. In some of the previously reported cases the strictures have undoubtedly been of tuberculous origin, but in many of them the aetiology is unknown (3). Some authors have expressed the view that the strictures were an atypical variant of Crohn's disease (2). In the present case there was fibrosis referable to an unspecific inflammation, but no granulomatous processes. It is uncertain whether the inflammatory changes were of a primary or secondary nature.

The megaloblastic anaemia must undoubtedly be ascribed to folate deficiency manifested by low folate concentrations in the serum with a pathologically high excretion of FIGLU substance in the urine. Additional folic acid was given only intermittently and as the body depots contain folate sufficient for only 4 months' consumption (13) it must be assumed that the patient had suffered from a severe folate deficiency. The results of the TRIFA test show that the folate deficiency was due to reduced absorption of folic acid from the intestine. The cause of this malabsorption is unknown. As in the presence of obstructive intestinal lesions the bacterial flora often changes in the direction of the milieu present in the colon (11) it is reasonable to assume that the pH of the stagnant intestinal contents was changed in the alkaline direction. If so the absorption of folic acid had been poor as it principally occurs at pH 6 (8).

As assessed by the Schilling test the intestinal absorption of vitamin B₁₂ was also reduced. It is assumed that intestinal stenosis with stagnation of the contents may lead to decreased absorption of intrinsic factor-bound vitamin B₁₂ because of the presence of an abnormal vitamin B₁₂-consuming intestinal flora (16). Even though the intestinal microflora was not studied in the present patient, the malabsorption of vitamin B₁₂ demonstrated may very well be due to such a mechanism.

The neurological defects ushered in by acro-

paresthesias and ending in incapacitating ataxia, are very similar to those of the subacute combined degeneration of the spinal cord which is seen in vitamin B₁₂ deficiency. Repeated determinations showed in fact decreased absorption of vitamin B₁₂, and as the neurological lesions principally developed during the period in which the patient failed to return for treatment with vitamin B₁₂ it would be tempting to explain the cord degeneration as being due to lack of this vitamin. However against this assumption there is the fact that subnormal serum levels of vitamin B₁₂ were not demonstrated at any time during the course of the disease. The neurological lesions can therefore scarcely be ascribed to vitamin B₁₂ deficiency. On the other hand severe folate deficiency was demonstrated on several occasions and in spite of oral administration of folic acid low serum concentrations of folate were found which is in agreement with the assumption of a reduced intestinal absorption of folic acid.

In general, folate deficiency is not assumed to result in neurological defects but nevertheless Reynolds et al. (15) often found folate deficiency in a series of patients with neurological disorders. However it must be presumed that the state of malabsorption has comprised not only vitamin B₁₂ and folic acid, but also other nutritional factors among which the lack of thiamine and niacin may have contributed to the development of the neurological disturbances. None of these vitamins were given in the period during which the neurological manifestations showed the most rapid progression. In addition, the ataxia had been preceded by a pronounced weight loss. It is therefore most probable that the subacute combined degeneration of the spinal cord was due to a deficiency of such nutritional factors as essential amino acids and certain members of the vitamin B complex. However by reducing the absorptive capacity of the intestine for these factors (7) folate deficiency may indirectly have contributed to the development of the neurological disturbances.

The malabsorption syndrome associated with multiple strictures of the jejunum carries a grave prognosis and may in spite of optimal substitution therapy with known vitamins lead to severe disability of the patient. The treatment of choice is

therefore as in the present case surgical relief of the jejunal strictures by which the intestinal malabsorption is to overcome.

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OSLER'S DISEASE WITH IMPAIRED ADHESION AND AGGREGATION OF PLATELETS

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Abstract In the investigation of haemostasis in 37 year-old women with Osler's disease the Ivy bleeding time proved to be prolonged. Platelet adhesiveness, measured both by Hellm and Salzman methods, was decreased and platelet aggregation was clearly reduced after addition of ADP but only slightly after collagen and epinephrine. Platelet factor 3 activity was normal, as was clot retraction. The therapeutic consequences of the platelet defect, including the risk of using drugs affecting platelet function are stressed. It is concluded that in cases of Osler's disease the possibility of functional disorders of platelets should be borne in mind.

Vascular malformations are sometimes accompanied by a systemic disorder of haemostasis with a latent or manifest haemorrhagic diathesis as a result. Such a disorder has been demonstrated in haemangiomas (7, 12, 13, 14, 15, 19, 1, 23). It may also occur in Osler's disease or hereditary haemorrhagic telangiectasia (17). The author has observed a case of this disease with impaired adhesion and aggregation of the platelets. The special therapeutic consequences that such a platelet defect might have were considered to justify a report of the case.

CASE REPORT

The patient was a 37-year-old Yugoslavian woman who had been living in Sweden for 6 years. No skin or mucosal lesions like the patient nor bleeding symptoms were known in the family. Two normal deliveries in 1958 and 1967. During the first pregnancy when the patient was living in Yugoslavia, she had had thrombophlebitis of the left lower leg. In 1966 she had had miscarriage. Menstruation had always been normal.

In 1969 the patient had painful bluish swelling of the tip of the right forefinger. She sought medical advice and an incision resulted in profuse bleeding that required suturing of the skin. Two years later she noticed bluish swelling in the proximal part of the right forefinger and in the right thumb wrist and elbow.

Two days before admission to hospital in Oct. 1971 she had found it difficult to open her mouth and to swallow. At the same time she noticed an increasing swelling in the right half of the floor of the oral cavity behind the mandibular angle and along the lower jaw. Neither then nor earlier had she ever had bleeding from the nose, gastrointestinal tract or lungs.

On admission to the Department of Internal Diseases physical examination disclosed that the patient was in good general condition without cyanosis or dyspnoea. The lower legs showed bilateral varices. Examination of the superficial lymph nodes, breasts, heart, lungs, abdomen and reflexes revealed nothing remarkable. The thyroid was moderately and diffusely enlarged. BP 140/80.

The oral and pharyngeal mucosa exhibited numerous red, scattered pin-point changes and, especially in the right buccal mucosa, a number of bluish, nodular lesions about 2x2-4x4 mm. Lesions of the latter type were also seen on the lower lip, the abdomen, on the distal part of the right forefinger and of somewhat larger size also over the right wrist and the right elbow. In the right part of the floor of the oral cavity there was tongue-sized fluctuant filling.

Routine laboratory investigations showed normal picture of the peripheral blood without anaemia or iron deficiency and a normal acid-base status. The oxygen saturation of Hb was 92-94%. Liver function studies disclosed normal values for serum enzymes and serum bilirubin. No proteinuria and normal sediment of freshly voided urine. ECG was normal. Chest X-ray showed an excavation about 2 cm long, possibly pressure erosion, on the dorsal side of the left fourth rib.

Table I Patterns of the coagulation and fibrinolytic systems

	1st examination	2nd examination	Normal range
Coagulation time (min)			
Glass	8		6-14
Plastic	19		1-32
Recalcification time (sec)	158		11-195
Factor VIII (%)	77		60-160
Factor IX (%)	96		60-160
One-stage prothrombin time (sec)	17.0		13-17
P&P (prothrombin + factors VII and X) (%)	87		80-120
Factor V (%)	72		80-120
Fibrinogen (g/100 ml)	0.26		0.20-0.40
Fibrinolytic activity on unheated fibrin plates			
Plasma (mm ²)	23	0	0-30
Resusp. erythrocyte precip. (mm ²)	56	29	0-70
Euglobulin clot lysis time (min)	120	300	100-300
Plasminogen (%)	91		70-130
Inhibitors of plasminogen activation (%)		113	60-140
FDP (μ g/ml)	25	11	0-5
Ethanol gelation test		Neg.	Neg.

COAGULATION STUDIES

Methods

The bleeding time was determined both by Duke's technique and by the method of Ivy as modified by Nilsson et al. (18). Platelet counts were made by the method of Björkman (4). Platelet adhesiveness was measured with the glass bead retention test, both according to (11) and to Salzman (20). Prothrombin consumption was tested according to Biggs and MacFarlane (3) retraction was determined according to Hart and Conley (10). Platelet aggregation was measured with Born's aggregometer (5) in the way described by Cronberg (6) after addition of ADP, epinephrine and collagen and the values were compared with those simultaneously obtained in a healthy volunteer. Other methods used were those formerly described (16).

RESULTS

Coagulation and fibrinolytic systems (Table I)

The coagulation factors appeared normal except for a slight reduction of factor V activity. The fibrinolytic system showed normal fibrinolytic activity, normal content of plasminogen and of inhibitors of plasminogen activation. Fibrinogen degradation products (FDP) in serum appeared however on both occasions. The ethanol gelation test was negative.

Platelet function (Table II) The bleeding time was normal according to Duke's method but

clearly prolonged according to Ivy's. The platelet count was slightly reduced at the first examination, but normal at the second. Platelet adhesiveness was decreased as measured by Hellens' method as well as by Salzman's. Determination of aggregation showed clearly reduced sensitivity to ADP but only slight reduction on addition of epinephrine and collagen. Clot retraction was normal as was the prothrombin consumption test.

DISCUSSION

Though the patient did not know of such changes in any of her relatives, the disease was probably Osler's disease or hereditary haemorrhagic telangiectasia. Neither her parents nor siblings had had bleeding symptoms. But vascular malformations of this type may not cause bleeding disorders until late in life or even not at all.

It is well known that in Osler's disease vascular malformations occur not only in the skin and the mucosa, but also elsewhere (2, 8, 22, and others). In the present case chest X-ray revealed an excavation of a rib which might have been due to pressure from an arteriovenous aneurysm. Owing to the haemostatic disorder it was decided to refrain from angiography. This disease may also be associated with liver cirrhosis (1, 9, 24). Labora-

Table II *Studies of the platelet function*

	1st examination	2nd examination	Normal range
Bleeding time (min)			
Duke	1	2	1-5
Ivy	25	22	6-1
Platelet number $\times 10^9/\mu\text{l}$	130	190	140-400
Platelet adhesiveness (%)			
Holten	15	15.5	17-33
Salzman		13.5	20-60
Prothrombin consumption test (%)	6		0-30
Platelet aggregation (Born)		Decreased	
ADP		Slightly decreased	
Epinephrine		Slightly decreased	
Collagen		Normal	
Clot retraction			

tory studies is the present case revealed no signs of liver disease which does not, of course exclude the possibility of cirrhosis.

In an earlier investigation Mucike using Borchgrevink's *in vivo* technique found platelet adhesiveness to be decreased in Osler's disease (17).

In the present case examination of the initial phase of haemostasis showed the Ivy bleeding time to be prolonged but the Duke bleeding time to be normal. Duke's method is less sensitive and a latent tendency to bleeding owing to disturbances of the initial phase of haemostasis is often not revealed by this technique. The prolongation of the bleeding time was not due to thrombocytopenia, as the platelet count was only slightly reduced at the first examination and normal at the second, but to a qualitative change in the platelets with decreased adhesiveness and aggregation. Examination of the aggregability of the platelets showed decreased sensitivity to ADP but an almost normal aggregation after addition of epinephrine and collagen. This means that the ability of the platelets to release ADP was practically normal i.e. the so-called release reaction was relatively intact. This is also supported by the fact that both the clot retraction, and the accessibility to platelet factor 3 measured with the prothrombin consumption test were normal.

In the coagulation phase factor V activity was slightly decreased, but otherwise the reaction in this phase was normal. The fibrinolytic system showed FDP in the serum but no signs of increased systemic fibrinolytic activity. FDP occur not only in primary fibrinolysis but also in fibrino-

lysis secondary to disseminated or local deposition of fibrin. A disseminated deposition of fibrin results in an increased consumption of platelets and coagulation factors with reduced values. In association with so-called reactive processes fibrinogen and factor VIII increase for which reason in such a situation normal values for these factors may be compatible with an increased consumption. In the present case however there were no signs of a reactive process. The normal values found for factor VIII and fibrinogen were therefore most likely not a manifestation of a relative decrease due to increased consumption despite a tendency to thrombocytopenia and decreased factor V activity at the first examination. The negative ethanol gelation test also argues against disseminated intravascular coagulation. The occurrence of FDP should therefore be regarded as an expression of local deposition of fibrin, presumably secondary to the bleedings.

It is obvious that disturbed platelet function considerably increases the risk of bleeding as associated with vascular malformations. In the present case it is true that bleedings resulting in hypovolaemia or anaemia had not yet occurred, but the submucous bleeding in the floor of the oral cavity if it had progressed further might have resulted in a life-threatening condition. Several of our most common analgetics and antiphlogistics, such as acetylsalicylic acid, indomethacin, phenylbutazone can reduce platelet aggregation by inhibiting the release reaction and thereby the release of ADP necessary for aggregation. In the present case the sensitivity to ADP was decreased, while the ability to release ADP was practically un-

affected. If the release reaction had also been inhibited by administration of a moderate amount of for example acetylsalicylic acid the risk of further bleeding would have been considerable.

In decreased platelet adhesion and aggregation especially in cases with haemangioma or telangiectasia, agents reducing platelet aggregation such as acetylsalicylic acid, indomethacin and phenylbutazone are contraindicated. This applies also to substances which influence platelet adhesion such as dipyridamol and what is of still greater practical importance dextran with a molecular weight of 70 000 (Macrodex®). In a situation with severe loss of blood and hypovolaemia other plasma expanders or fresh blood should be used. Transfusion of platelet concentrates may also be considered if available.

A platelet defect in Osler's disease is thus of both prognostic and therapeutic significance. Patients with this disease should therefore be examined also for such a defect. Ivy's method for determining the bleeding time covers platelet activity fairly well in the initial stage of haemostasis although occasionally cases with defective platelet function and normal Ivy time may occur. In practice, however, this examination alone is sufficient provided that it is performed *lege artis* and by experienced examiners. The coagulation phase is apparently of less interest in these cases unless liver function is impaired. In supplementary investigation of the haemostasis the fibrinolytic activity should also be determined but further investigation of the fibrinolytic system is presumably not necessary.

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ARTERIAL HYPERTENSION UNILATERAL CONTRACTED KIDNEY AND SUBCLAVIAN STEAL SYNDROME

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Abstract A case report is published concerning arterial hypertension, unilateral contracted kidney, subclavian steal syndrome, constriction of the right common carotid artery and hemolytic crisis. The renin concentration in the peripheral blood was found to be markedly increased. Following unilateral nephrectomy the renin concentration decreased to normal levels in the blood, but the hypertension remained. It is concluded that the elevated renin concentration was not the cause of the hypertension.

Subclavian steal syndrome is the name of a vascular anomaly characterized by a constriction or occlusion of the subclavian artery on the cardinal side of the left vertebral artery. The consequence of this abnormality is that the blood supply to the left arm is effected via the retrograde blood flow through the vertebral artery resulting in temporary cases of cerebral hypoxia with dizziness and coma. The aim of the present case report is a description of a very rare combination of arterial hypertension, unilateral contracted kidney with elevated concentration of renin in the serum, subclavian steal syndrome, constriction of the right common carotid artery and hemolytic anemia.

CASE REPORT

A 64-year-old woman was admitted to hospital on account of severe abdominal pains, nausea and vomiting. When she developed jaundice and elevated values of the liver function tests, the first diagnosis was an attack of gallstones. But later the Coombs' test was found positive, and the Hb concentration decreased after admission. Therefore it was concluded that the patient was suffering from hemolytic crisis (Table I).

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BP was measured with different results in both arms about 140/90 mmHg in the left and about 190/100 in the right. An arterial hypertension had been diagnosed about two years previously and the patient had been treated with methyldopa, hydrochlorothiazide and guanethidine for several months. In the ward she was treated effectively with hydrochlorothiazide and beta-sidolone, but when the BP decreased she developed syncope, convulsions and coma. These attacks and the different BP measurements in the left and the right arm caused suspicion of a vascular anomaly localized to the aortic arch or the arterial branches from the arch.

Aortic arteriography was performed and showed smooth stenosis of the right common carotid artery localized to the branching point of the carotid artery. The lumen was reduced to about half the normal diameter. Furthermore, there was constriction of the left subclavian artery and a retrograde filling of the left vertebral artery from the basis of the skull to the left subclavian artery. In this way the conditions for subclavian steal syndrome were proved.

The next problem was why the patient suffered from arterial hypertension measured in the right arm. About three years previously, pyelography had been performed in another hospital and hypoplastic left kidney was found. Therefore a new pyelography was performed, but no pyelogram appeared on the left side. In relation to the above mentioned aortic arteriography renal angiography was performed which showed normal artery supply to the right kidney but total occlusion of the left renal artery 1 cm from the aorta. The kidney was contracted, measuring 9x2.5 cm and was supplied with blood from collateral arteries. Therefore the most likely explanation of the arterial hypertension was: renovascular hypertension. The renin concentration was measured in the peripheral blood and was found enormously elevated (725 μ GU/ml, normal 6-60 μ GU/ml). This apparently was in agreement with renovascular hypertension. The measurements were performed in the Department of Clinical Physiology, Copenhagen County Hospital, Glostrup.

Following these diagnostic procedures the treatment of the patient was discussed with the vascular surgeons. It was decided to perform firstly plastic operation on the right common carotid artery and the left

Table 1 Hematologic values during the hemolytic crisis

Normal values given within parentheses

	Date of examination			
	22.11.71	25.11.71	11.12.71	15.12.71
Hb (g/100 ml) (11.7-15.7)	16.2	13.0	9.8	11.8
Hct (37-43)	49			35
Reticulocytes (<1%)				1.8
ESR (mm/h) (2-10)	87	120	93	102
Serum bilirubin (mg/100 ml) (0.2-1.2)	5.5	0.5	1.5	1.0
Serum haptoglobin (g/l) (0.60-3.2)	2.6			2.5
LDH (U/l) (100-370)	775			
Serum IgG (g/l) (7.4-14.2)	18.5			20.0
Serum creatinine (mg/100 ml) (0.8-1.2)	1.4	1.5	5.3	3.2

10-12.71 the serum contained markedly increased amounts of free autoantibody which reacted with enzyme technique. It was concluded that the patient suffered from an autoimmune hemolytic anemia and the direct Coombs test was positive.

clavian artery and secondly a left nephrectomy. These operations were successful and the patient is now free from the symptoms which led to admission to hospital and has no syncope and no convulsions. The renin concentration in the serum was normal after the nephrectomy (24 μ GU/ml) and the blood pressure decreased to normal levels (160/80 mmHg). Five months after the nephrectomy however it was again high 210/110 mmHg in the right arm and 190/110 mmHg in the left.

DISCUSSION

The patient history described is interesting with the following points:

1) Hemolytic crisis at the time of admission to hospital 2) Subclavian steal syndrome 3) Constriction of the right common carotid artery 4) Arterial hypertension 5) Unilateral contracted kidney with elevated levels of renin in the peripheral blood 6) Unchanged arterial hypertension in spite of nephrectomy of the contracted kidney and normal level of renin concentration in the peripheral blood after the nephrectomy

1) The hemolytic crisis. The hemolysis was Coombs-positive. The cause of the hemolysis is still uncertain but it is well known that prolonged treatment with methyldopa may provoke a Coombs-positive hemolysis. This patient had been treated with methyldopa for two years.

2 and 3) The anatomical defects which are described in cases of subclavian steal syndrome were found in this patient but they were complicated by a constriction of the right common carotid artery. These two bilateral constrictions may

explain the dramatic result of the effective anti-hypertensive treatment, because it led to an insufficiency of the blood flow to the two cerebral hemispheres with syncope, coma and neurological symptoms like those seen in cases of cerebral hemorrhage. The arterial hypertension was an essential condition for a normal cerebral function.

4, 5 and 6) The contracted left kidney with total occlusion of the renal artery was thought to be the cause of the arterial hypertension and this view was supported by the elevated concentration of renin in the peripheral blood. In cases of renovascular hypertension the renin-angiotensin system has been considered as the causal factor but no evidence exists for this view at the present time (1, 2, 4, 6). In the present case the renin concentration decreased to normal levels in the peripheral blood after the left nephrectomy but in spite of this the BP increased after the operation to almost preoperative levels. Therefore we may conclude that in our patient the renin-angiotensin system was not responsible for the arterial hypertension. Maybe the elevated level of renin in the peripheral blood was only a symptom of a destructive process in the left kidney with a liberation of renin to the peripheral blood. That enzymes are liberated to the peripheral blood in cases of organ-destructive processes is well known. It may take place in e.g. myocardial infarction and liver diseases.

The etiology of the multiple stenoses of the arteries was not clarified because only the removed left kidney was studied under the micro-

scope but not the contracted parts of the carotid and the subclavian arteries. The renal artery was constricted on account of endarteritis obliterans. Other possible causes of constriction of arteries besides atherosclerosis are polymyositis arteritica (5) or fibromuscular hyperplasia.

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HYPERTENSIVE CRISIS FOLLOWED BY ADRENOCORTICAL INSUFFICIENCY AFTER UNILATERAL ADRENAL PHLEBOGRAPHY IN A PATIENT WITH CUSHING'S SYNDROME

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Abstract A report is given of a patient with Cushing's syndrome who developed serious hypertensive crisis later followed by adrenocortical insufficiency after retrograde adrenal venography. It is concluded that this examination should be done only in selected patients who have to be kept under careful observation for hours following the procedure.

In both Cushing's syndrome and primary aldosteronism the preoperative differentiation between an adrenocortical tumour and a bilateral hyperplasia is important. The diagnosis of adrenocortical hyperfunction rests upon biochemical criteria. Demonstration of an adrenocortical tumour confirms the biochemical diagnosis and gives the side-localization. This makes selective surgery possible as opposed to bilateral adrenal exploration. A relatively large tumour seldom escapes detection by either retroperitoneal pneumography or by arteriography. The smaller adenomas, most often found in patients with primary aldosteronism, will however rarely be detected by these methods. In Conn's syndrome the differentiation between bilateral hyperplasia and unilateral tumour is of special importance as patients with bilateral hyperplasia, present in 14-30% of cases (2, 4), seldom benefit from adrenalectomy and should therefore not be subjected to surgical treatment (2). Unlike the case with Cushing's syndrome, biochemical differentiation between hyperplasia and tumour is not possible in patients with primary aldosteronism.

By means of retrograde adrenal phlebography (1, 10, 12), it is however possible to detect even small adrenocortical adenomas. Besides visualization of the adrenal gland the method enables a

comparison to be made between the concentrations of adrenocortical hormones in blood specimens taken from each of the two adrenal veins and from vena cava inferior (10-15). Although it may be technically difficult to perform selective catheterization of the adrenal veins, especially the right one, a comparison of blood concentrations of corticosteroids in one adrenal vein with that of vena cava inferior distal to the renal veins may give information of value for the differentiation of a unilateral tumour and a bilateral process (15).

Selective retrograde adrenal phlebography is however not without risks to the patient as is demonstrated by the following case report.

CASE REPORT

The patient, 64-year-old woman, was admitted to our department in 1972. In the last 3 years she had been treated with diet for mild diabetes mellitus. Her main complaint was intense backache and difficulties in walking due to an extreme osteoporosis of the spine with crush fractures of multiple vertebrae. Her BP was not elevated. She had not typical moonface but there was marked growth of facial lanugo hairs. The skin was thin and atrophic with subcutaneous haemorrhages but without striae. The prominent abdomen contrasted with her thin extremities, which showed muscular atrophy.

The clinical suspicion of Cushing's syndrome was confirmed by biochemical studies. Although the morning values for plasma cortisol concentration were not elevated, the diurnal variation was absent. Plasma cortisol determined fluorometrically (9) was at 8 a.m. within the range of 19.0-22.0 µg/100 ml. at 8 p.m. 17.8-19.8 µg/100 ml. The urinary excretion of 17-OHCS (11) was 19.9-28.8 mg/24 h (normal values for age and sex 2.0-10.0). The urinary excretion of 17-KS was only moderately increased.

Neither the plasma cortisol concentration at 8 a.m. nor the urinary excretion of 17-OHCS was suppressed to values lower than 16.8 $\mu\text{g}/100\text{ ml}$ and 18.5 $\text{mg}/24\text{ h}$ respectively even after dexamethasone 2 mg every 6 hours for 2 days. The plasma cortisol concentration increased sevenfold after ACTH administration while there was no response in metyrapone. Retroperitoneal pneumography performed to visualize the adrenals was inconclusive. Due to technical difficulties selective catheterization of the right adrenal vein was not successful. Blood samples from the left adrenal vein contained a high concentration of cortisol (approximately 900 $\mu\text{g}/100\text{ ml}$). A blood specimen taken from vena cava inferior also contained high plasma cortisol concentration (approximately 300 $\mu\text{g}/100\text{ ml}$) while the concentration in a cubital vein was 20.2 $\mu\text{g}/100\text{ ml}$. With the catheter in the left adrenal vein about 10 ml of the contrast medium, Isopaque® (Nyco) was immediately injected with increased rapidly until the patient felt slight discomfort in her left flank. The phlebography showed an enlarged left adrenal gland containing no visible tumour.

A few hours after the examination the patient complained of increasing pain in her left flank, with tenderness on palpation. Gradually a state of shock developed with anuria and mental confusion. Although her skin became cool moist and pale the BP was found unexpectedly to have risen from 120/80 to 10/1.5 mmHg, and the pulse was strong and rapid. Lowering the BP with α -blocking agents (phenolamine and chlorpromazine) brought the patient out of shock, but repeated administrations were necessary for the first 12 hours.

A urinary specimen sampled in the next morning indicated a markedly increased excretion of catecholamine metabolites. The excretion of vanillyl-glycolic acid was 35 $\mu\text{g}/\text{mg}$ creatinine (normal 1.5-4.0) which was increased over the next 5 days.

Although the patient improved initially weakness and perspired with a BP below 100/60 mmHg. On the third day after phlebography she became critically ill but improved promptly after administration of hydrocortisone. Unfortunately plasma for determination of corticosteroids was not sampled before treatment. After five weeks oral treatment the corticosteroids were withdrawn. The plasma cortisol concentration at 8 a.m. in the next morning was 2.7 $\mu\text{g}/100\text{ ml}$ and did not increase after ACTH (Synacthen, Depot® Ciba, 1 mg i.m. for 3 days). The plasma aldosterone concentration (16) was also low 6.0 $\mu\text{g}/\text{ml}$ (normal 25-150) and unresponsive to ACTH.

The treatment with corticosteroids had to be resumed. Repeated attempts to withdraw the treatment have been without success, the patient still needs cortisone 25 mg/day. Half a year after the adrenal phlebography plasma cortisol determined by competitive protein-binding assay (13) rose from ~ 7 to 1.0 $\mu\text{g}/100\text{ ml}$ after repeated injections of ACTH (Synacthen Depot). The urinary excretion of 17-OHCS rose from ~ 2 to 23.8 $\text{mg}/24\text{ h}$. The patient was treated with dexamethasone during the test.

COMMENT

The patient was a neglected case of Cushing's syndrome presenting with only a few of the typical clinical signs of the disease. Biochemically the probable pathoanatomical diagnosis was an adrenocortical adenoma while hyperplasia was the most likely radiological diagnosis. The high plasma cortisol concentration in the left adrenal vein indicated an increased cortisol production at this site. The high value found in "the inferior vena cava" probably means that this specimen had been sampled from the left renal vein into which the left adrenal vein empties and therefore was of no diagnostic value.

For this patient the differentiation between an adrenocortical tumour and bilateral hyperplasia was most important. A tumour would have had to be removed surgically but if the Cushing's syndrome was due to hyperplasia in this fragile patient we should first have tried pituitary irradiation instead of performing the usual bilateral adrenalectomy.

As it was the adrenal phlebography performed for diagnostic purposes turned out to be therapeutic. Eagan and Page (5) described a patient who developed adrenal insufficiency following bilateral adrenal venography. A cure of Cushing's syndrome after an apparent destruction of an adrenal adenoma following unilateral adrenal phlebography has also been reported (6). The same applies to a patient with primary aldosteronism (7). The mechanism is thought to be a haemorrhagic destruction of the adrenal cortex or of the cortical tumour due to the increased fragility of the veins in these patients. Bookstein et al (3) report on the frequent occurrence of intradrenal haemorrhage after adrenal phlebography. Although finding the method safe and reliable in other patients they consider the risk of serious complications to be so high that they do not recommend this examination as a routine in patients with adrenocortical hyperfunction.

In our patient the primary complicating mechanism has presumably also been an intradrenal haemorrhage. Judging both from the clinical symptoms and from the observation of an excessively high excretion of catecholamine metabolites in the urine the haemorrhagic lesion must have initially affected the adrenal marrow. It is also possible that the adrenal cortex has been directly

affected by the intradrenal haemorrhage. To explain the subsequent adrenal insufficiency we then must presume the left adrenal gland to have harboured an autonomous tumour suppressing the opposite gland.

Another possible mechanism is adrenocortical arteriolar constriction with bilateral adrenocortical necrosis due to the catecholamine efflux. Gold et al. (8) have reported on a hypertensive crisis following adrenal venography in a patient with pheochromocytoma. Hypertensive episodes due to adrenal phlebography have also occurred in patients with normal adrenal glands (12, 14) but in no instance known before has this mechanism been found responsible for the later development of adrenocortical insufficiency.

Because of the hazard of both a hypertensive catecholamine crisis and the risk of inducing adrenocortical insufficiency adrenal phlebography should only be performed in selected cases. The pressure and volume of dye injection should be controlled carefully and the patient kept under close observation for several hours following the examination. Development of a hypertensive crisis should be promptly treated with α -blocking agents.

Bilateral catheterization of the adrenal veins with sampling of blood for corticosteroid analyses but without contrast injections will most often be safe and informative procedure. Thrombosis of the iliac and of the adrenal veins however has been reported (1, 12).

At present there appears to be less need for the method of adrenal phlebography as adrenal photo-scanning following injection of ^{125}I -cholesterol may accurately differentiate between unilateral and bilateral adrenocortical hyperfunction (4, 17) and this method involves no risk to the patient.

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Announcements

The annual Ultrasound Conference will be held in Seattle Washington USA Oct. 5-10 1974. The American Society of Ultrasound Technical Specialists (ASUTS) will hold their meeting on Oct. 5 the Basic Education and Advanced Education sessions will be held on Oct. 6-7 and the American Institute of Ultrasound in Medicine (AIUM) scientific sessions on Oct. 8-10.

A postgraduate education course entitled Diseases of the liver will be presented by the Department of Medicine University of Miami School of Medicine Miami, Florida Nov 21-23 1974.

Director Professor L. Schiff MD

Location Playboy Plaza Hotel 5445 Collins Avenue Miami Beach, Florida 33140

Tuition \$150 physicians in training \$75 nurses \$50

Inquiries L. Schiff MD Department of Medi-

cine University of Miami School of Medicine P O Box 520875 Biscayne Annex Miami Florida 33152 USA

Again this year I am compiling case reports of allergic reactions to biting insects i.e. mosquitoes fleas gnats kissing bugs bedbugs chiggers, black flies horseflies sandflies deerflies etc. I am also interested in reactions to the Imported and Southern Fire Ants.

I would like physicians to supply me with case reports of those patients who have had reactions to such insects. Include in your reports the type of reaction and complications if any the age sex and race of the patient, the site of the bite(s) the season of the year the immediate symptoms the skin test results desensitization results if any and any associated other allergies. Send this information to Claude A. Frazier MD 4-C Doctors Park, Asheville NC 28801 USA.

EDITORIAL

RENAISSANCE OF A NEW FRONTIER IN OBESITY RESEARCH

All who have treated severe obesity by conventional therapeutic measures aiming at a negative caloric balance know how extremely difficult this is. This is also massively documented in the literature. The successful treatment of a patient with severe obesity defined as a maintained body weight within the limits of approximately normal is probably seen very seldom. After having tried to treat several hundred such patients the experience of the author is that when severe hyperplastic obese patients continue to reduce weight on an outpatient basis and approach the normal body weight, then there is some additional factor contributing to weight decrease and occult malignant disease should be remembered.

Why then, is severe obesity so difficult to treat in practice when the theory seems so easy? Are there factors which we still do not know sufficiently well and if so which may such factors be?

Energy balance

The first law of thermodynamics does of course apply also to severely obese subjects. Why then, do they not decrease to normal body weight in spite of high motivation and hard trying. This must be because they are not in a negative caloric balance signifying that caloric intake is sufficiently reduced in relation to caloric output or that the caloric output is smaller than anticipated. Usually in practice the main emphasis is placed on the caloric intake side the patient being more or less openly suspected to have a hidden caloric intake. This side of the equation is however easy to check: there are precise methods to determine the caloric equivalents of dietary intake and to assure that these calories have indeed been removed from the gastrointestinal tract.

The first factor on the caloric output side of the equation is of course physical activity. Obese subjects have been found often to be physically

inactive. There is however also another factor which is not directly associated with physical activity and which might cause a variation in caloric output. This is the caloric expense for other purposes than muscle work, and here the methodological difficulties are much greater than when caloric intake is measured. Particularly for long-time purposes these methods are very impracticable. This fact alone implies that it might be worthwhile to search for unknown factors which may explain the enigma of the failure of treatment of severe obesity.

Technical errors in measuring caloric output

Let us take some striking examples of the lack of precision of the methods applied for measurements of the caloric output due to other factors than physical activity. The accepted normal variation of $\pm 10\%$ in basal metabolic rate allows variation of oxygen uptake in absolute terms which may differ between comparable individuals by a factor of 2. This methodological error could account for several kg fat per year.

Another more genuine methodological difficulty is to estimate the caloric contents remaining in the body. A factor necessary to know when evaluating caloric balance. One kg adipose tissue contains at least ten times more calories than one kg lean tissue. Furthermore the caloric content of water is zero a fact which frequently seems to be forgotten in obesity treatment practice. Clearly then, body weight is not a sufficient measure one needs to know the exact amounts of body fat, body water and lean body weight. The possibility of obtaining exact measurements seems best for body water but is considerably less for body fat. A shift of weight of several kg from lean tissue to fat tissue corresponding to several tens of thousands of calories might well escape notice with the presently available techniques. This means that

experiments with energy balance measurements have to be performed over sufficiently long periods. Instead, unfortunately difficulties in measurement of the lost calories then appear. To measure heat production or oxygen uptake accurately over long periods seems in fact impossible with available techniques.

These methodological difficulties have probably prevented meaningful conclusions within this field. The clinical problem of obesity does not become smaller through the fact that weight increase is often a very slow process. In order to trace the reason for a weight increase of 5 kg per year one needs to be able to measure differences of the order of 100 calories per day. This is probably beyond the capacity of our presently available methods.

The Vermont study

Recent studies by a group in Vermont, USA (6) have in a most dramatic way brought this question into focus. These studies are impressive because the time axis is so long that the methodological problems mentioned are probably of minor importance. These investigators overfed young men with more than 5 000 calories per day for several months causing an excess of more than 1 mill calories in some cases. In spite of this magnificent overfeeding under controlled conditions some of the volunteers did not increase in body weight. This might be the reverse situation to the unsuccessful unhappy dieting patient, who does not reduce weight in spite of reduction of caloric intake. This points clearly to the fact that there is yet much to learn about energy regulation and within this field of ignorance the explanation of the enigma of the unsuccessful dieting patient may well be hidden. Let us therefore look into some more arguments within this field of obesity research which has in fact attracted the interest of several recent international congresses.

Old and recent information on caloric expenditure

The phenomenon of the subject who can eat whatever he wants without increasing in weight is well known. This empirical fact was recorded at the beginning of this century by Neumann (5) in studies on himself. Neumann noted that he could eat a wide range of calories but his body weight remained constant. It could be argued that he did not know his body composition before and after

the dietary periods. A shift in lean and fat body mass could explain missing calories. Furthermore his physical activity was not known. This is true but the observation periods were so long that these errors probably cancel out.

These were the first attempts to document the empirical observations. The Vermont study (6) and other studies referred to below then constitute a renaissance of an old frontier in obesity research.

Miller (3) reviewed some of these aspects in a recent congress in the following way. Careful studies of the caloric intake in a given population at a given body weight reveal that there is indeed a large variation. Actually some adults seem to manage to keep their body weight constant on the same caloric consumption as babies. The implication is according to Miller that caloric intake has comparatively little to do with the resulting body weight.

Another example taken by Miller to demonstrate the potential possibilities within this field is the variation of basal metabolic rate mentioned above. Obviously when the method is so crude large differences could easily be hidden during long periods. Nevertheless careful measurements of absolute oxygen uptake rates in different experimental situations have revealed small differences which when multiplied by the time factor become most important.

Basal metabolic rate is the oxygen uptake after sleep in the fasting and in resting state. Possible differences between different situations of interest are most likely minimized by these ambitions to standardize the situation examined. It seems more logical to perform the measurements after a standardized work load in the non-fasting state. Then the caloric expenditure is increased and potential differences between situations of interest should be accentuated. When performing these experiments Miller et al. (4) found that the energy output (oxygen uptake measurements) increases in subjects fed a hypercaloric diet, which might explain the lack of increase in body weight in some of these subjects. When measuring oxygen uptake over whole days it seems possible to explain satisfactorily the extra output of calories.

Is there a regulation of caloric expenditure

It is obvious that within this field very small differences in caloric intake and output play a large role over a year or more in terms of kg body fat.

It is therefore surprising that the caloric intake of different weight-constant subjects differs so vastly also under strictly controlled conditions during long periods. This leads Miller and others to believe that the regulation of body weight and body fat resides mainly on the caloric output side, the caloric intake playing a comparatively minor role. The output regulation is mainly due to the efficiency of handling calories, directing them to heat or to body fat.

Careful measurements have thus given experimental support to the idea that, in fact, there is probably a regulatory mechanism on the caloric output side. The next question is then how this can be explained on the biochemical and/or perhaps endocrine basis. Several theoretical explanations remain. These could be such phenomena as uncoupling of oxidative phosphorylation, or more specific energy leaking pathways of carbohydrate, protein and lipid metabolism. None has so far been convincingly demonstrated, but here too the possibilities of exact evaluation are limited.

This of course leaves us with the potential possibility that obesity is caused not so much by overeating but by a deficient system for disposing extra calories. *Luxus Konsumtion*, a term coined by the early German workers, signifying the potential to get rid of extra "luxus" calories, is not at work in subjects tending to become obese. They become obese by accumulating more ingested calories than do subjects without tendency to obesity. From this point of view accordingly it is normal to dispose of some ingested calories and abnormal to be too efficient in saving calories.

Adipose tissue cellularity and energy expenditure

The recent development of the cellular concept of adipose tissue and its relation to obesity seems to have a potential to create a better understanding of obesity. These observations may help mainly to distinguish different subgroups of obesity and therefore different possibilities for among other things, pathogenesis. The hyperplastic obese patients are those who are most difficult to reduce to a normal body weight by introduction of a negative

caloric balance (1). Furthermore when subjected to physical training, their weight reduction is abnormally slow (2). Weight decrease seems to cease at a certain fat cell size and seems actually possible to predict approximately when fat cell size is known at the start of treatment (1). These observations imply that there may be some association between adipose tissue cellularity and the possible caloric expenditure regulation.

The new information briefly reviewed here is still fairly preliminary in spite of the fact that the observations are old both empirically and in research. Nevertheless, it may open up means to solve a dilemma which most obesity treating doctors not to mention the poor obese patient have experienced as most frustrating, namely why some people increase in weight while others do not and why is conventional treatment of obesity very seldom successful.

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BOOK REVIEW

Minimal brain dysfunction. New York Academy of Sciences Vol 205 Edited by Felix F. de la Cruz, Bernhard H. Fox and Richard H. Roberts. 396 pages. \$30. New York 1973

Children with obvious handicaps, e.g. motor disabilities such as cerebral palsy syndromes, or intellectual defects such as mental retardation have successively been accepted as important groups for active habilitation programmes. Until recently probably much larger numbers of children with very slight deviations of different cerebral function have not been recognized more often been misconceived as "difficult" or "naughty" and have not received the necessary understanding and help in daily life and school. Some of these children have later in life been labelled as "lesional syndromes" by child psychiatrists and as minimal cerebral palsy or "minimal brain dysfunction" (MBD) by neuropsychiatrists. These children are mainly characterized by slightly retarded psychomotor development, defective servo-control of their motor function, impaired body image often very incapacitating perceptual difficulties, concentra-

tion disturbances with short attention span, emotional lability and hyperirritability and inability for abstract thought—but they have no or only very soft neurological abnormalities of conventional type.

This volume from NYAS collects a considerable amount of recent knowledge presented in different papers at a conference organized by the Academy in cooperation with the National Institute of Child Health and Human Development and the National Institute of Neurological Diseases and Stroke on March 20-22, 1972. Like all books of this type the level is uneven but by and large it gives a good insight into many of the multifaceted aspects of MBD. For the referee papers of particular clinical interest were "Follow up of infants at risk of minor brain dysfunctions" by the Prechtl group from Groningen and with a very strict scientific approach and "The natural life history of children with minimal brain dysfunction" by Dr Eric Denhoff from the Providence Center with his broad clinical experience of these problems over many years.

Bengt Hagberg Gothenburg, Sweden

ON A FAMILY WITH COEXISTENCE OF PHENOTYPES II AND III HYPERLIPOPROTEINEMIA

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Abstract Two brothers with xanthomata and coronary heart disease presented all the clinical and lipid-chemical characteristics of type III hyperlipoproteinemia. Preparative ultracentrifugation at density 1.006 revealed β -migrating lipoproteins in the supernatant fraction, and paper electrophoresis showed a "broad β band". Not only palpebral xanthelasmata and tendinous and tuberous xanthomata but also xanthochromia striata palmaris, as for only seen in types III and IV hyperlipoproteinemia, were present. However the family history indicated genetic type II, because 6 of the 8 sibs of the probands showed genuine type IIa hyperlipoproteinemia. The implications of these observations are discussed.

In 1965 Fredrickson et al. (5) described a method permitting better phenotyping of primary hyperlipoproteinemia on the basis of an improved paper electrophoretic separation of serum lipoproteins. This system seemed to lead to a simple classification into five types but the authors recognized that in type III hyperlipoproteinemia two subtypes could be distinguished: one characterized by increased amounts of Sf 12-20 lipoproteins and a broad band extending from the β to the pre- β position in paper electrophoresis, the other showing increased amounts of Sf 0-12 of the low density lipoprotein (LDL) fraction in combination with an increased Sf 20-400 fraction resulting in separate β and pre- β bands (5). It is claimed that these problem cases can now be satisfactorily identified by a modified classification (2), based on combined paper electrophoretic and ultracentrifugal analysis as a type IIb and type III with as differential diagnostic criterion, the presence or absence of "floating β lipoproteins" (4) whose presence is considered to be pathognomonic of type III. Substantial evidence has been presented that a family with

hyperlipoproteinemia can have members with type IIa (the "pure" hypercholesterolemia) and others with type IIb (4-10).

The present paper describes a family in which phenotypes II and III coexist, a situation which to the best of our knowledge has not been previously reported.

PATIENTS

Case 1 A 48-year-old man, first seen in Sept. 1965. He had suffered from angina pectoris for 4 years, during which period multiple purplish-brown tumours had developed on his elbows and yellowish streaks in the creases of the palms of his hands. He had been on a low-fat diet with restriction of alcohol. No other anamnestic data are available.

At examination he showed tuberous xanthomata on both elbows, xanthochromia striata palmaris (Fig. 1) prominent tibial tubera, and corneal arcs in each eye. BP 115/75 mmHg, ECG and chest X-ray normal. Weight 77 kg, height 167 cm.

Case 2 Brother of case 1 aged 45 years, examined in March 1966. For 15 years he had had yellow plaques on his eyelids and lumps in the tendons on the back of his hands. He had suffered from angina pectoris since 1960 and in Dec. 1965 he had had myocardial infarction, after which he had been on anticoagulants and low-fat diet, with complete restriction of alcohol and smoking.

He showed palpebral xanthelasmata (Fig. 2), tendinous xanthomata on the extensor tendons of both hands (Fig. 3), xanthochromia striata palmaris, tuberous xanthomata on one arm, and corneal arcs in each eye. BP 120/80 mmHg, ECG showed definite signs of myocardial infarction, chest X-rays were normal. Weight 93.3 kg, height 182 cm.

The relevant laboratory data of both patients, including blood count, fasting blood sugar, thymol turbidity, alkaline phosphatase, creatinine, protein electrophoresis, urinalysis and 24-hour urinary excretion of 17 ketogens, were all within normal limits.



Fig 1 Xanthochroa striata palmaris in patient 1



Fig 2 Xanthelasma mata palpebralis in patient 2.

The father of the patients, who had palpebral xanthelasmata, died suddenly at the age of 67. The mother died at 59 of cancer. A younger sister was reported to



Fig 3 Xanthomata tendineum in patient 2.

have diabetes mellitus and hypercholesterolemia. Unfortunately she was out of reach for examination.

Both patients were married. Each of them had four children, aged 6-26 years, all apparently in good health and completely normal on physical examination.

METHODS

Blood was collected after 12-24-hours fast, 22.5 ml whole blood being drawn into a 25 ml flask, containing 2.5 ml of standard citrate solution (33 g disodium citrate/l). For the preparation of serum a 10 ml blood sample was obtained by centrifugation for 5 min at 2500 rpm to precipitate the blood cells and then for 20 min at 4000 rpm to remove platelets. Serum was obtained by centrifugation of blood kept at 4°C for 4 hours. Lipoprotein fractions were obtained by combination of ultracentrifugation and precipitation (6, 13). The purified plasma and the serum lipoprotein fractions were extracted with methanol-chloroform mixtures. The sec-

Table 1 Lipid values

TL=total lipid, TS=total cholesterol, PL=phospholipids, FC=free cholesterol, CE=cholesterol esters, TG triglycerides, FFA=free fatty acids

	Pedigree no	Lipids (mg/100 cm ³)						% of total lipids					
		TL	TS	PL	FC	CE	TG	FFA	PL	FC	CE	TG	FFA
Case 1	II 1	1 676	533	486	153	646	381	10	29	9	38	23	1
Wife	II 2	631	208	215	57	252	101	6	34	9	40	16	1
Child	III 1	1 070	399	366	109	494	82	19	34	10	46	8	2
Child	III 3	1 073	391	374	91	510	68	30	35	9	47	6	3
Child	III 4	996	377	342	94	482	41	37	34	10	48	4	4
Case 2	II 3	2 018	541	474	170	603	722	22	23	8	32	37	1
Wife	II 4	776	243	278	63	302	95	36	36	9	39	12	5
Child	III 5	1 354	518	428	153	619	118	36	31	11	46	9	3
Child	III 6	709	206	206	57	255	170	21	29	8	36	24	3
Child	III 7	1 061	405	325	107	507	95	28	30	10	48	9	3
Child	III 8	819	305	277	71	388	53	20	34	9	49	6	

Table II. Lipid content of the various lipoprotein fractions

CH=chylomicrons VLDL=very low density lipoproteins, LDL=low density lipoproteins, HDL=high density lipoproteins

	Pedigree no	Sex	Age (y)	Lipoprotein lipids (mg/100 ml)				% of total lipids			
				CH	VLDL	LDL	HDL	CH	VLDL	LDL	HDL
Case 1	II 1	♂	48	83	647	780	166	5	39	47	10
Wife	II 2	♀	47	6	95	334	196	1	15	11	31
Child	III 1	♀	23	3	34	845	130	3	5	79	13
Child	III 3	♀	18	21	53	839	140		5	80	13
Child	III 4	♀	13	30	20	757	189	3		76	19
Case 2	II 3	♂	45	1.1	1 009	787	101	6	90	39	5
Wife	II 4	♀	44	16	109	465	186	2	14	60	4
Child	III 5	♂	21	41	136	1 001	176	3	10	74	13
Child	III 6	♂	1	7	41	333	128	1	34	47	18
Child	III 7	♀	14	21	116	785	138	2	11	74	13
Child	III 8	♀	6	8	37	631	148	1	4	77	18

Lipid material in the combined extracts was removed by Folch partition (3).

Determination of total lipids, phospholipids free and total cholesterol triglycerides, and free fatty acids were carried out as described elsewhere (13). Paper electro-

phoresis was performed according to the method of Lees and Hatch (8).

RESULTS

Both patients showed xanthochromia striata palmare, which is a two-dimensional analogue and precursor of the xanthoma striata palmare (1). In the literature they are sometimes designated as planar xanthomata of the palms but this term is confusing (1).

Plasma lipid analysis (Table I) revealed in both

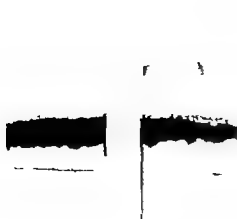


Fig. 4 Broad β band on paper electrophoresis in patients 1 and 2.

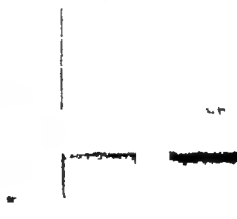


Fig. 5 β moving VLDL ("floating β ") on paper electrophoresis in patient 2. VLDL to the left, LDL+HDL to the right.

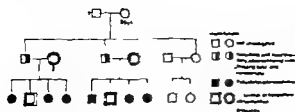


Fig. 6 Pedigree of the family

brothers a combined hypercholesterolemia and hypertriglyceridemia with a cholesterol/triglyceride ratio of about 1.35. Paper electrophoresis showed a broad β band (Fig. 4). In the preparative ultracentrifuge (Table II) the LDL fraction (density 1.006–1.063) was moderately increased, the very low density lipoprotein (VLDL) plasma fraction (density <1.006) greatly increased. The plasma VLDL, isolated by preparative ultracentrifugation at density 1.006, migrated paper electrophoretically in the β region (specific for 'floating β ' lipoproteins) (Fig. 5).

Six of the 8 children presented a type IIa hyperlipoproteinemia (Fig. 6). Their serum lipid and lipoprotein values are given in Tables I and II. All 6 have increased LDL and normal VLDL concentrations, increased plasma cholesterol and a chol/TG ratio > 1. Floating β s were not found. Paper electrophoretically they all showed an increased intensity of the β band and a normal pre β band.

DISCUSSION

According to the WHO classification (1) type IIb hyperlipoproteinemia is chemically characterized by an increase in both LDL and VLDL. On electrophoresis they show distinct β and pre β bands. Type III hyperlipoproteinemia should have one unequivocal criterion: the presence in the plasma of an abnormal VLDL with β electrophoretic mobility. Furthermore, paper electrophoresis usually shows a broad β band extending from the β to the pre- β position. Both of our probands fulfil these criteria for the diagnosis of type III hyperlipoproteinemia. Floating β lipoproteins were demonstrated in their serum, and a 'broad β band' was found on paper electrophoresis. The quantitative lipoprotein and plasma lipid analyses supported this diagnosis. Furthermore xanthochromia striata palmarum, so far only seen in type III and type IV

patients (11) occurred in the palmar creases of these probands.

Our investigations show, however, that 6 of the 8 sibs of the probands are heterozygous type IIa. Consequently both probands must be carriers of the genetic trait responsible for type II, both mothers of the IIa siblings being normal.

The following possibilities can now be considered. (a) All of the presented patients are genetically type II. In the probands the 'floating β ' may be an epiphenomenon, as is suggested by the fact that it was found only at the first examinations and disappeared during diet therapy. The children might also present phenotype IIb or even III later in life. (b) In this family two genetic traits coincide: the dominant type II and the incomplete dominant type III (4, 10) which is not yet expressed in the siblings. The xanthoma pattern in the probands favours this hypothesis. A conclusive choice between these possibilities cannot be made yet.

The present study, however, emphasizes that the relationship between genetic classification and lipoprotein phenotype is not a single and specific one. The occurrence of phenotypes III and IV in the same family has already been reported (4, 9). This study brings unequivocal evidence for the presence of phenotypes IIa and III in a single family. Also Hazzard et al. (7) concluded from a study of survivors of myocardial infarction that β VLDL may not be a specific marker for a single and distinct lipid disorder. Contrary to a previous assumption (2) it appears also from our data that the presence of β VLDL in a patient and a first degree relative may not be pathognomonic for a definite genetically determined type III lipoprotein disorder.

Note at the correction

We were finally able to examine the blood of child III 2, aged 26. The lipid and lipoprotein values proved to be within the normal range: Lipids: TL 851 TS 289 PL 272 (32%) FC 68 (8%), CE 383 (45%), TG 26 (1%), FFA 76 (3%). Lipoproteins: CH 9 (1%), VLDL 153 (18%), LDL 44 (52%), HDL 747 (79%).

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SERUM LIPID AND LIPOPROTEIN CONCENTRATIONS IN A NORWEGIAN POPULATION SAMPLE

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Abstract Serum lipids and electrophoretically determined lipoprotein concentrations have been analyzed in 168 healthy subjects (118 males and 50 females) belonging to two different age groups. An increase in serum lipid and lipoprotein levels with age was seen. No difference was found between the concentrations in young males and females. Higher concentrations of β - and pre β -lipoproteins and lower level of α -lipoproteins were found in males than in females in the age group 40-59. Serum cholesterol values were determined in a series of 828 males and 382 females of different ages (15-69 years). In males the mean cholesterol values increased from 197 mg/100 ml in the age group 15-19 to 280 mg/100 ml in the age group 40-49. Means close to the latter age value were found also in the higher age groups. In females, mean cholesterol value decreased throughout life from 208 mg/100 ml in young females to 337 mg/100 ml in those aged 60-69.

There appears to be no general agreement on the "normal values" for plasma lipids or lipoproteins. For the purpose of establishing reference values for further genetic studies in hyperlipoproteinaemic subjects and their families lipid and lipoprotein analyses were conducted in several groups of persons.

MATERIAL AND METHODS

Subjects Four groups (A-D) of healthy individuals were randomly selected and all subjects in these groups were interviewed and subjected to clinical examination. Symptoms or signs of cardiovascular disease were not revealed in any of the subjects. No person used drugs known to influence lipid or lipoprotein levels. All blood samples were drawn after an at least 10-hour fast, and serum lipids and lipoproteins were studied.

Research fellow the Norwegian Council on Cardiovascular Diseases.

This composition of the four groups was as follows: group A: 25 males, 18-24 years old (mean 21); group B: 25 females, 21-24 years old (mean 22); group C: 93 males, 40-59 years old (mean 48); group D: 25 females, 40-59 years old (mean 48).

In two additional groups (E and F) of 15-69 years old persons the concentration of serum cholesterol only was investigated. Group E consisted of 828 men and group F of 382 women, sampled at voluntary health control. Within each group the members of individuals with muscular and with sedentary work were approximately equal. Most, but not all subjects were in the fasting state when blood was collected. No individuals were excluded from either of these two groups.

Sample samples Blood was collected by venepuncture and allowed to clot for 2-4 hours, and the serum was then pipetted off. Lipoprotein electrophoresis was performed within two days on small aliquots kept at 4°C, whereas the major part of the serum was kept at -28°C until the lipid analyses could be performed.

On samples from series A-D simultaneous determinations of serum cholesterol and triglycerides were carried out in an AutoAnalyzer® by the methods of Block et al (2) and Kessler and Lederer (14) respectively. Serum samples from some of the males in group C were kindly provided by O Mowé MD A/S Borregaard, Sarpsborg. Serum total cholesterol was determined on samples from groups E and F by the method of Carr and Dreikler (5).

Cholesterol (Eastman Organic Chemicals) was dissolved, crystallized 4 times and used as cholesterol standard. Trioleine (Applied Science Laboratories) served as triglyceride standard, and the values are given relative to the molecular weight of 885.5. This gives results 3% above the equivalent molar concentration of triglycerides.

Phospholipids were determined as lipid phosphorus (8) and the values converted to phospholipids by multiplication by 25. Total serum lipids were calculated from the cholesterol, triglyceride and phospholipid values (6).

The methodological error expressed as the coefficient of variation between duplicate determinations on the same samples, was found to be 3% or 6-10 mg/100 ml

Table I Serum lipid and lipoprotein-lipid values (mg/100 ml) in groups A-D

Mean \pm S.D. range 95th percentile (in italics) and coefficient of variation (%) (within parentheses)

Group	Sex	Age (y)	N	Cholesterol	Triglycerides	Phospholipids	Total lipids	β -lipoproteins	Pre- β -lipoproteins	α -lipoproteins
A	δ	18-24	25	180 \pm 4 145-230 224 (13)	85 \pm 40 30-165 164 (47)	10 \pm 29 168-280 275 (14)	565 \pm 76 450-790 775 (13)	305 \pm 66 205-450 435 (22)	102 \pm 35 55-180 178 (34)	158 \pm 79 85-10 204 (18)
B	η	1-24	25	185 \pm 26 150-35 245 (19)	70 \pm 28 25-135 131 (40)	218 \pm 24 170-255 252 (11)	570 \pm 66 445-665 664 (1)	296 \pm 64 170-435 425 (22)	103 \pm 28 40-155 154 (77)	171 \pm 35 85-205 202 (20)
C	δ	40-59	93	256 \pm 54 150-435 335 (21)	133 \pm 57 38-462 303 (43)	248 \pm 37 175-338 323 (15)	794 \pm 151 516-1 233 1 115 (19)	429 \pm 106 200-785 595 (25)	164 \pm 95 40-574 389 (58)	187 \pm 47 110-315 273 (25)
D	η	40-59	25	258 \pm 39 188-340 340 (15)	100 \pm 37 55-172 169 (37)	255 \pm 32 197-318 316 (13)	743 \pm 113 535-1 007 975 (14)	379 \pm 74 225-525 519 (70)	111 \pm 45 60-225 209 (41)	253 \pm 49 180-340 340 (19)

 $p < 0.01$ $p < 0.001$ for difference in values between males and females of the same age

for both cholesterol methods 5% or 4-8 mg/100 ml for triglycerides and 5% or 4-7 mg/100 ml for phospholipids. The two methods for cholesterol determination were compared by analysing 80 different sera by both methods. The mean values from the two series were very similar (279.6 and 279.9 mg/100 ml). The triglyceride values obtained with the present method compare well with those obtained by Carlson and Lindstedt (4), as by the exchange of ten serum samples with different triglycerides.

Lipoprotein electrophoresis is Quantitative serum lipoprotein electrophoresis was performed in agarose gel as earlier described (13). The values for the lipid part of the lipoprotein complex (lipoprotein-lipid) of each class of the lipoprotein were estimated by multiplying the value obtained for total lipids by the relative amount of dye uptake in each fraction.

Low density lipoprotein (LDL) quantification. The ap-

proximations given (9-11) for LDL-cholesterol concentrations without the use of preparative ultracentrifugation were employed as follows: LDL-cholesterol = Total cholesterol - very low density lipoprotein (VLDL)-cholesterol - high density lipoprotein (HDL)-cholesterol where VLDL-cholesterol is taken as total triglycerides divided by 5 and HDL-cholesterol is arbitrarily given as 45 mg/100 ml.

Another measure of LDL-cholesterol was obtained by multiplying the electrophoretically determined β -lipoproteins by the cholesterol content, 41.5% (3) in this lipoprotein.

These two estimates were compared by regression analysis and the correlation coefficient was determined.

Statistical analysis. Comparisons between averages were performed by Student's *t*-test and the regression line was obtained by the method of least squares.

Table II Serum total cholesterol concentrations (mg/100 ml) in groups E and F

Group E (males)				Group F (females)			
Age (y)	N	Mean \pm S.D.	95th percentile	N	Mean \pm S.D.	95th percentile	
15-19	56	197 \pm 33	265	58	208 \pm 32	271	
20-29	84	230 \pm 40	314	37	221 \pm 40	295	
30-39	207	259 \pm 39	341	28	247 \pm 3	300	
40-49	198	280 \pm 50	383	134	284 \pm 58	360	
50-59	171	287 \pm 50	373	109	322* \pm 59	421	
60-69	112	282 \pm 48	384	50	337** \pm 61	448	
Total	828			382			

** $p < 0.001$ for the difference between males and females of the same age.

Table III Comparison of serum total cholesterol concentrations (mg/100 ml) among four Scandinavian male population samples aged 40-59 years

	No. of subj.	Mean \pm S.D.	% of population with cholesterol higher or equivalent				
			300	325	350	Mean + 2 S.D.	400
Present series							
Group C	93	256 \pm 54	18	9	5	4.3	—
Group III	369	284 \pm 50	26	20	10	4.0	1.6
Berge & Nicolaysen	6 357	270 \pm 51	26	13	6	3.5	1.1
Carlson & Lindstedt							
Group I0	914	280 \pm 50	32	18	11	3.5	2.0

RESULTS

The results of the lipoprotein and lipid determinations for groups A-D are given in Table I and for groups E and F in Table II.

All lipid and lipoprotein concentrations showed an increase with age in both sexes. Serum cholesterol reached its highest mean level in males (group E) in the age group 40-49, whereas it continued to increase in females (group F) up to a mean value of 337 mg/100 ml in the age group 60-69. Serum cholesterol concentrations above 400 mg/100 ml were found in 1.5, 9 and 70% of the females

(group F) in the age groups 40-49, 50-59 and 60-69.

No striking differences in cholesterol concentrations were found between males and females in the younger age groups in the small series (groups C and D). The difference between the two sexes with respect to the cholesterol value was however highly significant in the age groups higher than 50 years in groups E and F. Highly significant differences ($p < 0.001$) were also found in mean cholesterol concentrations between males in the selected and unselected series C and III and between the females in groups D and F. The highest levels were found in the unselected series E and F.

The percentage of males 40-59 years of age with cholesterol concentrations higher than certain values commonly used to define hypercholesterolaemia (300, 325, 350 and 400 mg/100 ml) are given in Table III and compared with results from two other Scandinavian investigations (1, 4).

The distribution of some of the lipid and lipoprotein concentrations are illustrated in Figs. 1-4. The distribution of triglyceride values in group C was distinctly skewed (Fig. 2). All these individuals were fasting and none had chylomicronaemia as judged from appearance of their sera and from electrophoretograms. Eight of the 12 subjects with triglyceride concentrations higher than 10 mg/100 ml were reinvestigated. Their triglyceride levels were high also in the new samples.

To evaluate the information obtained by lipoprotein quantification, the results in group C were further analysed. None of the 93 subjects had elevated phospholipid or total lipid levels without increase in either cholesterol or triglyceride concentrations, using the 95th percentile as the upper limit. By the same criterion (95th percentile) ele-

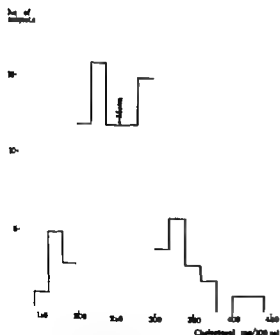


Fig. 1 Distribution of serum cholesterol values in group C (93 males, 40-59 years).

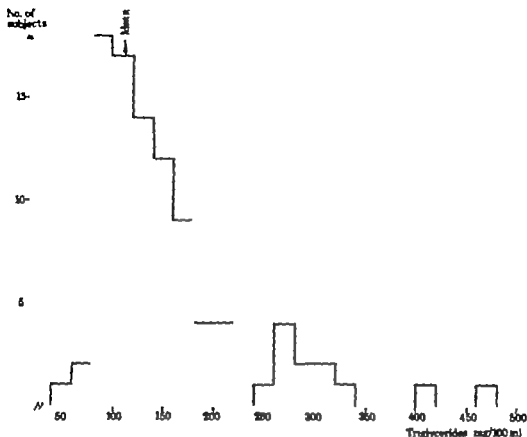


Fig. 2 Distribution of serum triglyceride values in group C (93 males, 40-59 years)

β - or pre β -lipoprotein concentrations were in 6 of 8 subjects with increased cholesterol or triglyceride levels, and a further 3 of the remaining 85 males had moderate rises in β - or pre- β -lipoprotein values without elevation of cholesterol or triglycerides.

The two estimates of LDL-cholesterol concentrations generally showed good agreement in the 168 subjects. The correlation coefficient was found to be 0.84 and the regression equation was $Y = 0.763X + 30.2$ where Y is the electrophoretically determined LDL-cholesterol and X the cholesterol value computed from the plasma triglyceride and cholesterol concentrations.

Somewhat lower values were found for LDL-cholesterol determined by the electrophoretic method. The largest differences between the results from the two methods were seen with plasma with increased amounts of triglycerides and pre- β -lipoproteins. The 95th percentile for LDL-cholesterol by the "calculation method" based on serum triglycerides and cholesterol concentrations was found

to be 179, 156, 279 and 267 mg/100 ml in groups A, B, C and D.

DISCUSSION

The serum lipid concentrations found in this study showed skew distributions, most pronounced for triglycerides in males 40-59 years old (group C). Logarithmic transformation normalized the distribution of the lipid and lipoprotein levels with the exception of that of triglyceride concentration.

The lipid levels found in this study are in good agreement with those from other Scandinavian studies (1, 4, 7, 15). The lipoprotein lipid levels, as obtained by densitometry of agarose gel electrophoretograms, are similar to those reported by Dyerberg and Hjorne (7) and are also in good agreement with those found in ultracentrifugation studies after correction for the apoprotein content of the different apoproteins (10, 12, 16). This confirms the validity of the method employed.

For all serum lipid and lipoprotein concentrations an increase with age was observed in groups

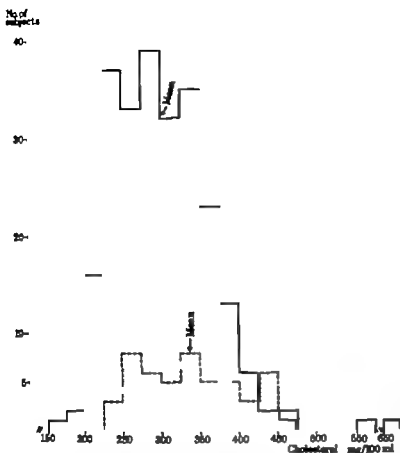


Fig. 3 Distribution of serum cholesterol values in 293 females (40-69 years) from group F — 90 females, 60-69 years — 243 females, 40-59 years.

A through D. For cholesterol the increase was also observed throughout in group F. The rise in cholesterol levels did not continue in males above 50 years of age (group E) a finding which also has been reported by other workers. Significant differences in lipid and lipoprotein concentrations between the youngest males and females (groups A and B) were not seen but were evident between groups C and D where the triglyceride β - and pre- β -lipoprotein levels were higher in men and the α -lipoprotein level was higher in women.

The expected rise in β - and pre- β -lipoprotein levels with increasing cholesterol and triglyceride concentrations respectively was confirmed but very few subjects had elevated β - or pre- β -lipoprotein fractions without concomitant rise in either cholesterol or triglyceride levels.

The wide range observed for cholesterol and other serum lipid concentrations could possibly be narrowed by quantification of separate lipoprotein fractions. However the coefficients of variation for the separate lipoproteins were higher or

similar to those observed for serum cholesterol and triglycerides.

The definition of a "biologically normal" upper limit for lipid and lipoprotein concentrations is not possible from the present data. The 95th percentile for each age and sex group may be utilized for an operational definition of normality despite the small numbers of subjects in several of the groups studied. However the limitations of this pragmatic method must be kept in mind.

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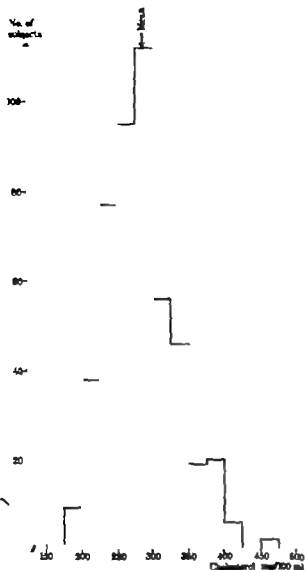


Fig. 4 Distribution of serum cholesterol values in 481 males (40-69 years) from group E

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THE DIABETES SURVEY IN BERGEN NORWAY 1956

A Ten-year Follow up of an Epidemiologic Study and a Study of Blood Sugar Values Related to Sex, Age and Weight

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Abstract. A follow-up study of 209 persons examined in the diabetes survey in Bergen in 1946 is discussed. The material was divided into five groups on the basis of laboratory findings from 1946. *Latent diabetic.* In 1946 diabetes was strongly suspected in most persons in this group. The follow-up study showed possible diabetes in 1 of 3 persons. The average age of the men was 69, of the women 75. They used insulin and 3 persons nondiabetics. In the remainder the disease could be kept under control by diet. *Diabetic complication of moderate degree.* were observed in 7 persons. The mortality during the observation period was 60% for both sexes. Cardiovascular disease was found in a large percentage of the deceased. *Borderline cases.* In this group diabetes was found to have developed in 8 of the 41 persons examined. The average age of the men was 65, of the women 70. The diabetes of this group had easily controllable diabetes. The diagnosis is still uncertain in 46% of the group. *Glycosuria non-diabetic.* Diabetes was found in 5 of the 41 persons examined. *Screened positive non-diabetic.* Diabetes was found in 2 of the 36 persons examined. *Normal maternal.* Diabetes was, somewhat surprisingly, found in 7 of the 68 persons examined. As the attendance at the follow-up survey was only 70% it is possible that certain degree of selection had taken place. It can therefore hardly be assumed that the prevalence of diabetes in this group is representative of the development of diabetes in the constituency of Nygård during the observation period. The follow-up study has showed considerable reduction in glucose tolerance during the observation period, the average levels of both fasting blood sugar and 2-hour blood sugar after administration of glucose showing clear increase in all diagnostic groups.

The first epidemiologic study of the prevalence of diabetes in a population group was made in 1946 by Wilkerson and Krall (13). Blood and urine were

analysed and 40 known diabetics and 30 new cases were found in the group of 3516 persons examined, i.e. 0.9% diabetics in all. This survey revealed that diabetes is more common than earlier assumed and that there is a large number of undiagnosed cases in the general population.

Wilkerson and Krall also found a group of 118 individuals with abnormally high blood sugar levels who could not be classified as diabetics. A follow-up after 4 years (14) showed 17 new cases (14.4%) in this high blood sugar group. In a control group of 225 persons 4 diabetics were found (1.8%).

Since 1946 diabetes surveys have been carried out in a number of countries, particularly in America (1) but also in Europe (5). The prevalence of diabetes in these surveys has varied between 0.4% and 5.5%, the number of new cases varying from 0.22% to 1.17%.

In a follow-up study after 7 years of 800 persons with high blood sugar levels Fisher and McDonald (3) found 8% new diabetics. Only in very few cases (3, 9, 12) have studies been made to see how the disease develops in the newly discovered cases and of developments in persons with high blood sugar levels or glycosuria who could not be diagnosed as diabetics.

In 1946 a diabetes survey was made in Nygård constituency Bergen Norway (6) in connection with a mass X-ray screening. In 1966-68 follow-up study was made of the persons who were found to have high blood sugar levels and/or glycosuria at the first examination, and of a normal maternal

RÉSUMÉ OF 1956 SURVEY

A total of 796 men and 4645 women above the age of 18 attended the 1956 mass X-ray screening, of whom

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Table 1 Mortality during the observation period 1956-1966-68

Diagnosis in 1956	Sex	N	Deaths		Age at death (y)	
			n	%	Range	Mean
Latent diabetics	♂	28	17	60	47-81	68
	♀	42	25	60	68-89	81
Borderline cases	♂	41	14	34	47-84	73
	♀	41	15	37	62-87	75
Non-diabetics Glycosuria	♂	37	2	6	43-46	45
	♀	17	1	6	74	74
Screened positive	♂	26	3	11	57-76	67
	♀	28	7	25	58-86	72
Normal	♂	39	4	10	55-93	78
	♀	58	4	7	62-66	64
Total		357	92	23		

2273 men and 3657 women took part voluntarily in the diabetes survey.

On the basis of predetermined screening levels for blood sugar or glycosuria, diabetes was suspected in 510 persons (259 men, 251 women). Of these 19 men and 21 women were earlier known diabetics under treatment. The remaining 240 men and 230 women were examined further with glucose tolerance tests 100 g glucose per os. A "normal material" of 194 persons was subjected to the same tests, to provide a basis of comparison for evaluation of glucose tolerance. The following criteria were used in grouping the material.

Diabetes was diagnosed if the fasting blood sugar was higher than 120 mg/100 ml or the 2-hour blood sugar higher than 180 mg/100 ml. The diagnosis of borderline case was made if the 2-hour blood sugar was between 150 and 180 mg/100 ml. If the 2-hour level was below 150 mg/100 ml the diagnosis of non-diabetic was made. Glycosuria was not considered necessary for a diagnosis of diabetes. The final diagnoses after glucose tolerance tests in 1956 were as follows: latent diabetics 28 men and 42 women, borderline cases 41 men and 41 women, non-diabetics 150 men and 122 women. There were 21 men and 25 women who did not attend the examination. Of the 510 screened positive 129 had glycosuria, of whom 44 were diagnosed as non-diabetics.

METHODS USED IN 1956 SURVEY

Urine sugar was tested with Clinotest tablets. Capillary blood sugar was analysed by Sørensen's modification of Dreywood's anthrone method (11). The height and weight of all attending were measured.

The anthrone method is suitable for mass surveys, but requires concentrated sulfuric acid and is thus extremely unpleasant. In 1966-68 the Hagedorn-Jensen reduction method for blood sugar determination was employed. A parallel study carried out in 1956 with the anthrone and Hagedorn-Jensen methods gave the result,

after statistical analysis, that the fasting blood sugar levels obtained by the anthrone method were on an average 10.09 mg/100 ml higher and the 2-hour blood sugar levels in tolerance tests 11.71 mg/100 ml higher than by the Hagedorn-Jensen method. In recent years it has become more and more common to determine blood sugar by the glucose-oxidase method, which gives "true blood sugar" (Erikson et al (2)) found in a parallel study that the Hagedorn-Jensen method gives levels 18 mg/100 ml higher than the glucose-oxidase method.

METHODS USED IN 1966-68 FOLLOW-UP

Plan of study

Urine was examined for sugar with Tes-Tape for ketone bodies with Ketosticks, and for albumin with Albusticks.

Capillary blood sugar was determined by the Hagedorn-Jensen method. The following examinations were made: 1) Fasting blood sugar. 2) Morning urine was examined for sugar, ketone bodies and albumin. 3) Tolerance tests with 100 g glucose per os were carried out in all persons not being treated for diabetes. Double specimens of capillary blood were taken after 1 and 2 hours, the mean value of the analyses being entered as the result. 4) Clinical examination. All those attending the blood sugar survey were given a clinical examination by one of us (E. A. or S. R.) with a view to cardiovascular disease, neuropathies, retinopathies and nephropathies. All attendants were weighed.

Death certificates were obtained for all who had died during the observation period.

Criteria for diagnosis of diabetes

In a publication issued in 1963 (1) an expert committee appointed by the WHO gave criteria for the diagnosis of diabetes mellitus. The Committee stressed the desirability of standardizing examination methods and diagnosis criteria in order to reach a better basis of comparison for the results of various studies.

Table II. *Causes of death (main (M) and second (S) diagnosis) based on notified diagnosis to the Registrar of the Bergen Public Health Office*

Diagnosis in 1956	Total mortality	Diagnosis		Cardiovascular diseases		Morbus subita		Other	
		Diabetes		diseases		Morbus subita		Other	
		M	S	M	S	M	S	M	S
Latent diabetes	♂ 17 ♀ 25	5	5	12	1	2		3	3
Borderline cases	♂ 14 ♀ 15		1	11	2	1		8	3
Non-diabetics	♂ 2 ♀ 1		3	6	4			4	2
Glycosuria	♂ 3 ♀ 7			1		1		9	
Screened positive	♂ 4 ♀ 4			2	1			3	1
Normal	♂ 4 ♀ 4			3		1		5	
				1		1		2	

As to diagnosis, the Committee laid most emphasis on 2-hour blood sugar after peroral tolerance test, giving the following levels for capillary "true blood sugar": Levels below 120 mg/100 ml are normal. If levels are higher than 140 mg/100 ml, the diagnosis of diabetes can be made. Levels between 120 and 139 mg/100 ml are defined as borderline levels. It is strongly recommended that this group (borderline cases) shall be re-examined at regular intervals. The Committee added: "When a higher level is found in a person over 45 years of age, and especially over 65 the Committee considered that the other clinical data—family history, physical examination, etc.—should provide the main guides to the diagnosis and treatment needed, since population-study data becoming available make it clear that aging is associated with diminishing glucose tolerance (i.e. higher blood-sugar curve).

Classification of material from the follow-up study in 1966-68 has been made in accordance with WHO diagnostic criteria, blood sugar levels found by the Flagerö-Jensen method being converted to "true blood sugar".

Mortality during observation period 1956-1966-68

There was a considerable mortality within the material (Table I), among the latent diabetics 60% both for men and women.

Mortality among borderline cases was also high, 34% for men and 37% for women. Statistical analysis of these figures (carried out by L. Olsson-Rodman) showed that mortality rate was above normal in both latent diabetics and borderline cases, but that the difference was not statistically significant. In this connection it may be mentioned that Wilkström and Krall (14) found considerably higher mortality among the known and newly diagnosed diabetics. Copies of death certificates are obtained for all deaths (Table II). Among the latent diabetics diabetes was given as the main cause of death for 5 women, and as a contributory cause for 4 women and 5 men. Among the borderline cases diabe-

tes was given as second diagnosis for 1 man and 3 women. From this it will appear that 30% of the dead females and 19% of the dead males in the groups latent diabetics and borderline cases were known to have diabetes at the time of death.

Cardiovascular disease dominated the list of causes of death. Among latent diabetics cardiovascular disease was given as the main cause of death in 23 of 41, and as a contributory cause in 11. In other words, in 35 of the 42 latent diabetics (83%) cardiovascular disease was known to be present at the time of death. If morbus subita is included, the figure rises to 38 of 47 (80%).

In the group borderline cases cardiovascular disease was listed as the main cause of death in 14 of 29 cases, and as a contributory cause in 6. Twenty of the 29 borderline cases (69%) were thus known to have cardiovascular disease at the time of death. If morbus subita is included the figure reaches 76%.

MATERIAL OF FOLLOW UP STUDY

IN 1966-68

It was our intention to examine all survivors among the 70 latent diabetics, 82 borderline cases and 54 persons with glycosuria from the 1956 survey.

The group screened positive non-diabetics numbered

Table III. *Survivors at follow-up study 1966-68*

Diagnosis in 1956	Males	Females	Total
Latent diabetes	11	17	28
Borderline cases	27	26	53
Non-diabetics			
Glycosuria	35	16	51
Screened positive	23	21	44
Normal	35	54	89
Total	131	134	265

Table IV Attendance at follow-up study in 1966-68

Diagnosis in 1946	Attendants (n)		Age of males (y)		Age of females (y)	
	Males	Females	Range	Mean	Range	Mean
Latent diabetes	9	14	61-74	69	55-83	73
Borderline cases	1	20	45-86	65	5-80	70
Non-diabetics						
Glycosuria	30	11	25-71	57	3-71	57
Screened positive	18	18	38-77	55	28-87	46
Normal	79	39	25-86	40	40-89	47
Total	107	102				
Both sexes	209					

777 persons in the original material. Of these each fifth person was picked out by random sampling so that 54 of the group took part in the follow-up study. Half of the 'normal material' group from 1946 was selected in a similar manner giving a group of 97 for the follow-up study.

Table III shows the distribution of the survivors in 1966-68 in the various diagnostic groups, and Table IV the attendance at the follow-up study by sex and age. Of the 265 unmooned 709 presented for examination

RESULTS OF FOLLOW UP STUDY IN THE DIFFERENT DIAGNOSTIC GROUPS

Table V shows the fasting blood sugar levels and Table VI the 2-hour blood sugar levels for men and women in the different diagnostic groups in 1956 and 1966-68. The levels found by the anthrone method in 1946 have been converted to Hagedorn Jensen levels.

Table V Fasting capillary blood sugar levels in 1956 and in 1966-68 (Hagedorn Jensen method)

		Capillary blood sugar (mg/100 ml)							Total
		<120	120-129	130-139	140-149	150-159	160-180	>180	
Males	Latent diabetes	1946		4	2		1		9
		1966-68	5		1	1	1	1	9
	Borderline cases	1946	19	1	1				1
		1966-68	16		1				17
	Non-diabetics Glycosuria	1946	29	1					30
		1966-68	27	1		1	1		30
	Screened positive	1946	18						18
		1966-68	18						18
	Normal	1946	28	1					29
		1966-68	25	1	1				27
Female	Latent diabetes	1946	7	2	1	1	1		14
		1966-68	7	1	2	1	1	2	14
	Borderline cases	1946	20						20
		1966-68	17	1	1	1			20
	Non-diabetics Glycosuria	1946	11						11
		1966-68	11						11
	Screened positive	1946	18						18
		1966-68	17		1				18
	Normal	1946	39						39
		1966-68	36		1				37

Determination failed in 1 persons.

Table VI Glucose tolerance test with 100 g glucose per hour: capillary blood sugar levels in 1946 and in 1966-68 (Hagedorn-Jensen method)

		Capillary blood sugar (mg/100 ml)							Total
		<120	120-139	140-159	160-179	180-199	>180		
Men									
Latent diabetes	1946				1	2	8	9	
	1966-68		1	1			5	2	
Borderline cases	1946		1	3	8	1	1	1	
	1966-68	1			1	5	5	12	
Non-diabetics									
	Glycosuria								
	1946	19	9					28	
	1966-68	13	9			1	1	24	
Screened positive	1946	14	4					18	
	1966-68	11		3	1	1	1	16	
Normal	1946	17	7	1	1		1	27	
	1966-68	14	4	1	1	5	1	26	
Females									
Latent diabetes	1946					6		11	
	1966-68				1	1	10	12	
Borderline cases	1946		2		5	4	1	12	
	1966-68	3	1		1	4	4	13	
Non-diabetics									
	Glycosuria								
	1946	7	4					11	
	1966-68	3		4	1		1	11	
Screened positive	1946	11	3	4				18	
	1966-68	6		1	4	1		12	
Normal	1946	26	5	3				34	
	1966-68	9	4	6	7	5	1	28	

Determination failed in 1 persons.

Determination failed in 1 person.

Latent diabetes

Seven men of 9 examined and all 14 examined women were now diagnosed as positive diabetics. In male persons the diagnosis of diabetes could not be confirmed.

The age within the group was high (Table IV). In most cases the disease could be classified as a relatively mild old-age diabetes. Two used insulin and 3 personal antidiabetics. In the remainder the disease had been kept under control by diet. Clinical examination showed moderate diabetic complications in 4 men and 3 women (angiopathy, polyneuritis, retinopathy, nephropathy, cataract and arteriosclerosis obliterans).

Borderline case

This is the group described by WHO's Committee as 'border-line state' in which a relatively high development of diabetes can be expected. The

final diagnosis at the follow-up examination showed that 4 men of 11 examined and 4 women of 20 examined were positive diabetics. Nine men and 10 women were still classified as borderline cases and 8 men and 6 women as non-diabetic. Moderate neuropathy was found in 1 woman. The follow-up study confirms the expectation of a high rate of development in this group and that persons with blood sugar level in the borderline area between definitely normal and pathologically high should be kept under supervision.

Glycosuria

Four men of 30 examined and 1 woman of 11 examined had developed diabetes. One male person is classified as borderline case. Twenty-five men and 10 women have been diagnosed as non-diabetics. No diabetic complications have been demonstrated. The study indicated that findings of glycosuria should not be brushed aside easily.

Table VII Height/weight ratio in 1956 and in 1966-68 (Nativity tables)

		Underweight (%)				Overweight (%)				
		>20	20-16	15-10	<10	<10	10-15	16-20	21-25	>25
<i>Males</i>										
Latent diabetics	1956						3	2		2
	1966-68				3	2	3			1
Borderline cases	1956				4	7	3	1		4
	1966-68		2	2	3	9		2		3
Non-diabetics Glycosuria	1956		1	4	11	10	4			
	1966-68	3	1	4	8	12	2			
Screened positive	1956	1		1	5	9	1	1		
	1966-68	1		2	4	8	2			1
Normal	1956		1		10	12	1	2	2	1
	1966-68	1		1	10	12	1	2	2	
<i>Females</i>										
Latent diabetics	1956				1	4	3		1	5
	1966-68				3	4	3			4
Borderline cases	1956			1	6	3	1	3	2	4
	1966-68		2	1	4	7		2	2	2
Non-diabetics Glycosuria	1956				4	3	1			
	1966-68				4	1	1	1	2	2
Screened positive	1956	1		2	6	6	1	1	1	
	1966-68		1	3	5	3	1	2	2	
Normal	1956			2	12	16	2	2		1
	1966-68	2	2	1	13	12		1	2	4

Determinations missing in 3 persons.

Determinations missing in 1 person.

blood sugar levels are normal. Persons with glycosuria should be examined at regular intervals.

Screened positive

This group showed abnormally high blood sugar levels at the first screening, but glucose tolerance tests gave normal levels in 1956.

The result of the follow-up survey showed that of 18 men and 18 women examined 2 men had developed diabetes. The others were non-diabetics. No diabetic complications were demonstrated.

Normal material

In 1956 the normal material was selected by random sampling among those who had shown a negative reaction to the chosen screening levels. In 1966-68 as earlier mentioned half of the group was selected for follow-up examination.

Of 79 men examined 4 were diagnosed as diabetics, 4 were classified as borderline cases and

21 as non-diabetics. Of 39 women examined 3 were diagnosed as diabetics, 6 were classified as borderline cases and 30 as non-diabetics. This result is astonishing. The age of the group however is high (Table IV). Attendance at the follow-up examination was only 70%. The possibility that this was to some degree a selected portion of the group cannot therefore be disregarded.

For this reason it can hardly be assumed that the prevalence of diabetes in this group is representative of the development of diabetes in the constituency of Nygård during the observation period, especially if a comparison is made with the group screened positive non-diabetics in which the prevalence would be expected to be higher than in the normal material.

Height/weight ratio during observation period

The height/weight ratio in 1956 and in 1966-68 for men and women appears from Table VII. No major changes took place in the height/weight

Table VIII. Fasting mean capillary blood sugar level (mg/100 ml) (Hagedorn-Jensen method) with S.D. in 1956 (x) and in 1966-68 (y)

	Latent diabetes	Borderline cases	Non-diabetics		
			Glycosuria	Screened positive	Normal
Males (N)	9	19	30	18	39
Mean (x)	129.0 ± 7.0	103.4 ± 2.3	91.1 ± 1.9	93.9 ± 1.2	91.2 ± 1.8
S.D.	21.1	9.9	10.2	5.1	9.8
Mean (y)	135.1 ± 14.9	109.0 ± 2.7	101.8 ± 3.6	102.3 ± 2.4	101.7 ± 2.6
S.D.	44.8	11.6	19.6	10.2	13.9
Percentile for x/y	<p 90	>p 99	>p 99	>p 90	<p 99
Females (N)	14	20	11	18	39
Mean (x)	121.5 ± 7.3	98.6 ± 1.9	91.3 ± 1.9	90.4 ± 1.5	88.1 ± 1.1
S.D.	27.4	8.3	6.4	6.4	7.3
Mean (y)	132.8 ± 11.8	106.1 ± 3.4	96.6 ± 1.7	103.5 ± 2.7	98.6 ± 1.7
S.D.	44.3	15.4	5.8	11.3	10.6
Percentile for x/y	<p 90	<p 90	>p 95	<p 90	<p 90

ratio during the observation period. A slight displacement towards a higher weight can be seen in females in the groups glycosuria, screened positive and normal.

Changes in glucose tolerance during observation period

A number of studies have shown that glucose tolerance decreases with age (4, 6, 7, 10). This was also clearly apparent when the diabetes ma-

terial from 1956 was analysed as there was a definite statistical increase with age in the average levels of both postprandial blood sugar and blood sugar after glucose tolerance tests. This reduction in glucose tolerance was present both in normal weight and overweight groups and can thus not be ascribed to the general weight increase with age.

The changes in glucose tolerance for the material in the years 1956-1966-68 appear in Tables

Table IX. Glucose tolerance test with 100 g glucose per os 2-hour mean capillary blood sugar level (mg/100 ml) (Hagedorn-Jensen method) with S.D. in 1956 (x) and in 1966-68 (y)

	Latent diabetes	Borderline cases	Non-diabetics		
			Glycosuria	Screened positive	Normal
Males (N)	7	20	30	18	28
Mean (x)	184.0 ± 9.7	150.9 ± 3.4	108.3 ± 4.1	108.6 ± 4.2	114.7 ± 4.1
S.D.	25.7	15.4	22.2	17.8	21.5
Mean (y)	228.7 ± 24.5	153.1 ± 8.9	144.0 ± 6.3	118.8 ± 7.3	126.3 ± 7.8
S.D.	64.8	39.9	34.4	31.1	41.5
Percentile for x/y	<p 90	<p 90	<p 90	<p 90	>p 99
Females (N)	13	19	11	18	37
Mean (x)	200.4 ± 12.1	148.6 ± 3.6	111.4 ± 3.3	116.2 ± 3.1	115.7 ± 2.9
S.D.	43.7	15.7	13.3	13.3	17.7
Mean (y)	238.4 ± 20.4	158.5 ± 9.3	129.0 ± 5.3	134.7 ± 4.9	138.7 ± 4.7
S.D.	73.7	40.7	17.7	20.9	28.3
Percentile for x/y	<p 90	<p 90	<p 90	p 90 < <p 95	>p 99

VIII and IX Table VIII shows the average levels for fasting blood sugar and Table IX the average levels for 2-hour blood sugar with S D for men and women in the various diagnostic groups for the years 1956-1966-68. The anthrone method levels from 1956 have been converted to Hagedorn-Jensen levels. Both mean value of fasting blood sugar and 2-hour blood sugar show an increase in all diagnostic groups both for men and women. The difference is statistically significant for fasting blood sugar for male borderline cases men and women with glycosuria and men in the normal material. For 2 hour blood sugar the difference is statistically significant for the men and women in the normal material.

The study shows that the fasting blood sugar value increased in all groups in the course of a ten-year period by an average of 9 mg/100 ml (5-13 mg/100 ml) and the 2-hour blood sugar value by an average of 18 mg/100 ml (3-44 mg/100 ml). The difference was greatest in the group latent diabetes in which the increase was 44 and 38 mg/100 ml for men and women respectively. If this group is disregarded the increase in the remaining groups is 13 mg/100 ml (3-23 mg/100 ml). At the same time we see that the S D values have increased in the course of the observation period. The 1956 survey showed that glucose tolerance decreases with age. The follow-up study shows

for the age groups the material represents. Increase during a ten-year period will be approximately 9 and 13 mg/100 ml for fasting and 2-hour blood sugar values respectively.

DISCUSSION

Hayner et al (4) have demonstrated that with increasing age blood sugar levels 1 hour after oral glucose loads rise steadily by approximately 13 mg/100 ml each decade from 113 mg/100 ml in the thirties to 173 mg/100 ml in the eighties. We know neither the reason for the reduction in glucose tolerance registered with increasing age nor the effect it may have on the development of other diseases. Many theories have been proposed. Hayner et al consider that the reduction affects everyone and is thus simply an effect of old age.

The large dispersion of levels for both fasting and 2 hour blood sugar in our material and the fact that dispersion increased during the observation period, argue against the theory that the

reduction in glucose tolerance affects everyone. Eriksen et al (7) have carried out a health survey which included a glucose tolerance test of 410 70-year-olds. According to WHO's criteria 7.8% of this material were diabetics, 19% latent diabetics and 17.3% borderline cases. 55.5% had normal glucose tolerance tests. Eriksen et al found that the frequency of abnormal glucose tolerance tests increased with increasing occurrence of other abnormal examination findings. Among healthy 70-year-olds 79% had normal glucose tolerance. This study does not appear to indicate that there is a general reduction of glucose tolerance with age and Eriksen et al assert that WHO's criteria for the diagnosis of diabetes should also apply to the elderly. Kaufman et al (7) also concluded from their material that the reduction of glucose tolerance is not evenly distributed in the population, and can thus not be ascribed to age. They suggested that the reduction might be due to the occurrence of an inherited disposition to diabetes in the population. This is a question which should be resolved as it would be of decisive importance for our criteria for the diagnosis of "old-age diabetes and thus also for treatment. Keen et al (8) have shown that there is a clear relationship between reduced carbohydrate tolerance and frequency of cardiovascular complications.

Is there a connection here or are we dealing simply with two parallel processes both due to increasing age? Only long-term studies of a relatively large population group can solve this problem.

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ESTIMATION OF SERUM THYROTROPIN (TSH) AND STIMULATION WITH THYROTROPIN RELEASING HORMONE (TRH) IN THYROID DISEASES

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Abstract. Serum TSH has been estimated using radioimmunochemical method in 128 controls: 19 ± 17 $\mu\text{U/ml}$ (mean \pm S.D.) in 97 hyperthyroid patients: 1.5 ± 1.5 $\mu\text{U/ml}$, and in 40 patients with primary hypothyroidism: 116 ± 72 $\mu\text{U/ml}$. After stimulation with 200 μg TRH a positive response ($\Delta \text{TSH} > 2.0$ $\mu\text{U/ml}$) was found in 58 of 60 controls but only in one of 56 hyperthyroid patients. In 23 hypothyroid patients combined with the TRH test an exaggerated TSH response was found. Four clinical euthyroid patients with Hashimoto thyroiditis had moderately elevated serum TSH and an increased response to TRH whereas 9 euthyroid patients with diffuse goitre did not differ from the control group. During antithyroid treatment of 66 patients serum TSH was elevated only in half of the patients with subnormal serum thyroxine and normal in almost all cases with normal serum thyroxine. In 34 patients with primary hypothyroidism treated with L-thyroxine serum TSH was elevated in all with subnormal serum thyroxine and was not normalized until serum thyroxine levels were within the upper part of normal range. Estimation of serum TSH is of major diagnostic value in primary hypothyroidism and valuable aid in the control of treatment of hypo- and hyperthyroidism. The TRH stimulation test is of value in the diagnosis of hyperthyroidism.

The TSH secretion of the pituitary is stimulated by thyrotropin-releasing hormone (TRH) which is secreted from the hypothalamus. In 1969 TRH was identified (3, 31) as a tripeptide pyroglutamyl-histidyl-prolinamide and shortly thereafter its synthesis became practicable (8, 9). The effect of TRH on the release of TSH is inhibited by thyroxine and triiodothyronine (5, 33) and the effect of TRH on the TSH response therefore dependent on a normal function both of the thyroid and of the pituitary gland. Thus, several studies have shown a lack of TSH response after TRH stimulation in some patients suffering from pituitary disorders (7, 13, 18) as well as in patients with hyperthyroidism (14, 18, 28). We have studied the diagnostic value of serum TSH estimations before and after stimulation with TRH in controls and in patients with thyroid diseases. Furthermore serum TSH has been followed during antithyroid treatment and treatment of hypothyroidism.

MATERIAL

Since 1965 it has been possible to measure serum thyrotropin (TSH) by radioimmunochemical methods (26, 36). Using these methods different investigators have found considerably elevated values of TSH in patients with primary hypothyroidism. Although the TSH concentration in patients suffering from hyperthyroidism has generally been found to be lower than in healthy persons, in most laboratories it has not been possible on the basis of a single TSH estimation to distinguish between hyperthyroidism and euthyroidism (12, 14, 26, 27, 30, 36).

The control group consisted of 128 persons without thyroid diseases, 67 men and 61 women aged 18-87, average 49.3 years. The TRH stimulation test was performed in 60 of them (28 men and 32 women aged 16-87, average 46.5 years). The group with untreated hyperthyroidism consisted of 97 patients, 85 women and 12 men aged 14-88, average 51.8 years. The TRH test was performed in 56 of these patients (51 women and 5 men aged 14-84, average 44.8 years). In 66 patients with hyperthyroidism serum TSH was measured during long-term antithyroid treatment. For 41 patients this was the first attack, for 25 the second or more attack of hyperthyroidism. The patients had been treated from weeks to 10 years, mean 8 months, and none thyroid hormone. Serum TSH was determined 1

Table 1 Serum TSH ($\mu\text{U/ml}$) in controls and patients with hyperthyroidism, hypothyroidism and diffuse non-toxic goitre

	<i>n</i>	S.D.	Range	95% limits	
Euthyroid	128	1.9	1.7	0-11.4	0-4.8
Hyperthyroid	97	1.5	1.5	0-8.7	0-3.5
Hypothyroid	40	11.6	7.2	26-340	36-290
Diff. se non-toxic goitre	14	1.3	1.5	0-5.0	-

patients with primary hypothyroidism (35 women and 5 men aged 13-83, average 59.6 years) and 43 of these patients (21 women and 22 men aged 13-83, average 59.5 years) were followed with TRH. In 34 patients suffering from primary hypothyroidism serum TSH was studied during treatment. Serum TSH was also measured in 14 patients with diffuse non-toxic goitres and in 9 of them the TRH test was performed. Finally 4 women with Hashimoto thyroiditis were examined.

METHODS

The thyroid function was evaluated on the basis of serum thyroxine according to the method of Murphy, T_4 -resin uptake (Trisorb, Abbott) and the 4 and 24 h ^{125}I uptake in the thyroid gland, possibly with TSH stimulation or triiodothyronine suppression of the ^{125}I uptake. Serum TSH was determined by radioimmunoassay with a modification of Wide's solid phase technique (39). Human TSH from National Pituitary Institute, National Institute of Health, Bethesda, USA, labelled with ^{125}I (The Radiochemical Centre, Amersham) according to Hiter and Greenwood (15).

Labelled TSH was purified during a passage through G 100 Sephadex columns (A. 9760, Pharmacia). The antibody against human TSH was obtained from National Institute of Health. After the immunoglobulins had been precipitated with 18% Na_2SO_4 they were fixed to cyanobromide-activated microcrystalline cellulose (Merck). Human TSH 68/38 from National Institute for Medical Research, London, was employed for the standard. TSH was dissolved in 0.05 M phosphate and 0.15 M NaCl buffer with 1% bovine albumin (Behring Werke) with pH 7.5. To 0.1 ml of the standards 0.1 ml O-serum was added. The O-serum was collected from euthyroid persons who had been suppressed with 120 μg T daily for 7 days. To 0.1 ml of serum samples 0.1 ml of the buffer was added. About 40 000 cpm of ^{125}I -labelled TSH was added both to the serum samples and to the standards. Finally 1.0 ml of the antibody suspension in the above mentioned buffer with 0.5% Tween 20 (Koch-Light Laboratories) and 1 U human chorionic gonadotropin (HCG) (Wellcome) was added. HCG was added in order to saturate antibodies to TSH, LH, FSH and HCG. After rotation of the mixture for 3 days at room temperature

the tubes were centrifuged and the supernatant removed by suction. The centrifugate was washed 3 times with 0.1 ml 0.30 M NaCl with 0.5% Tween 20 and finally counted in a well scintillation counter. All analyses were done in duplicate. A standard curve and three known serum samples were run every day.

The lowest serum TSH value which was significantly different from 0 was 1.0 $\mu\text{U/ml}$ and the recovery of added human TSH to 10 different sera was 98% \pm 10%. The coefficient of variation as found by repeated determinations of the same sample 20 times on consecutive days was within the normal range 15% and for the sample with high TSH content 12%.

The TRH stimulation test was performed in the morning. The patients were not fasting. A permanent drip with 0.9% saline was applied and at zero time 200 μg TRH was injected intravenously. Blood samples for TSH estimation were drawn at 0, 20, 60 and 120 min. TRH was supplied by F. Hoffman-La Roche & Co. and Novo Industri A/S. No difference between the preparations was found.

Statistics: Student's *t*-test was used except for materials including less than 25 persons. In these cases Wilcoxon's test was employed.

RESULTS

The TSH values were below 4.9 $\mu\text{U/ml}$ in 95% of the 128 controls (Table 1). In 36% serum TSH could not be detected ($<1.0 \mu\text{U/ml}$). No differences between the sexes could be demonstrated (Table II). The relation to age was investigated by dividing the controls into three age groups: below 40 years (average 26.4), 40-59 years (average 49.5) and above 60 years (average 68.8) (Table II). Serum TSH levels were found to increase with age though a significant difference was demonstrated only between men below 40 and above 60 years ($p < 0.05$).

The mean maximal response of serum TSH to TRH is given in Table III. In all but two of the controls a rise in serum TSH (i.e. maximal response $>2.0 \mu\text{U/ml}$) could be demonstrated. The maximal value of serum TSH was reached in 43 persons 20 min following the TRH injection, in 1 person after 60 min and in 5 persons the maximum was not reached until after 120 min. However the actual difference between the TSH values at 60 and 120 min in these persons was very small. No difference in the response to TRH between the sexes was found (Table II). Age dependent changes in the TSH response to TRH were evaluated by dividing the persons studied into three groups of mean age 24.3, 50.3 and 71.5 years respectively (Table II). A significant

Table II Serum TSH ($\mu\text{U/ml}$) and mean maximal TSH response (Δ Max TSH) to 200 μg i TRH in control

Age (y)	Males		Females	
	Baseline TSH	Δ Max. TSH	Baseline TSH	Δ Max. TSH
	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
<40	3 1.2 \pm 1.3	14 6.3 \pm 9	14 1.6 \pm 1.5	11 7.4 \pm 3
40-59	4 1.9 \pm 1.3	7 8.3 \pm 9	4 1.9 \pm 1.3	8 7.5 \pm 5.0
≥ 60	20 2.3 \pm 1.6	7 1.9 \pm 1.6	3 5 \pm 1.9	13 8.9 \pm 7.5
Total	67 1.8 \pm 1.8	28 4.9 \pm 3.5	61 0 \pm 1.6	32 8.0 \pm 5.6

age-dependent decrease in the serum TSH response to TRH was demonstrated only in men ($p < 0.05$). Only a slight correlation between the basal TSH value and the maximal response to TRH was found ($r = 0.27$ $p < 0.05$). The reproducibility of the TRH test was estimated by repeating the TRH stimulation with an interval of at least one week in 7 persons. The mean rise in serum TSH 20 min after TRH injection was 4.5 $\mu\text{U/ml}$ with S.D. of 1.6 $\mu\text{U/ml}$ calculated from the duplicate estimations. The corresponding values at 60 and 120 min were 3.0 ± 1.3 and 1.1 ± 1.0 $\mu\text{U/ml}$. Judged by the Wilcoxon test for paired data no significant differences between the TSH responses were found ($p > 0.10$).

In 97 patients with untreated hyperthyroidism mean serum TSH was found to be significantly lower ($p < 0.05$) than in the control group. The results are seen in Table I. In 47% of the patients serum TSH was below 1.0 $\mu\text{U/ml}$. The TRH test was performed in 56 of the hyperthyroid patients (Table III). Only one of these responded with an increase in serum TSH of more than 2.0 $\mu\text{U/ml}$ after 120 min.

In all the patients with primary hypothyroidism serum TSH was found to be considerably elevated

and an exaggerated response to TRH was found in all patients in whom this test was performed.

The patients suffering from Hashimoto thyroiditis were clinically euthyroid and had serum thyroxine within the normal range (mean value 6.7 $\mu\text{g/ml}$). Serum TSH was moderately elevated in all of them and after TRH stimulation a considerable increase in TSH was observed. Serum TSH was found to be normal in the patients with diffuse non-toxic goitre and the TRH test showed normal response of serum TSH in these patients.

The results from the patients during long term antithyroid treatment are shown in Fig. 1. In cases with subnormal serum thyroxine levels serum TSH was elevated only in 57%. In patients with normal serum thyroxine values serum TSH was found to be normal except in 4 cases. Serum TSH estimations from 34 patients with primary hypothyroidism receiving L-thyroxine treatment are shown in Fig. 2. Serum TSH was found to be elevated in all cases with subnormal serum thyroxine values, among cases with normal serum thyroxine below 9 $\mu\text{g}/100$ ml serum TSH was elevated in 73% but only in 16% with serum thyroxine above 9 $\mu\text{g}/100$ ml.

Table III Serum TSH ($\mu\text{U/ml}$) in response to 200 μg i.v. TRH in controls and patients with hyperthyroidism, hypothyroidism, Hashimoto thyroiditis and diffuse non-toxic goitre

		Δ Max. TSH		p^*
		Baseline TSH (Mean \pm S.D.)	Mean \pm S.D. Range	
Euthyroid	60	2.0 \pm 1.8	7.1 \pm 4.8 0.6-23.7	<0.001
Hyperthyroid	46	1.1 \pm 1.3	0.3 \pm 1.0 0-7	0.12 $p > 0.05$
Hypothyroid	23	125 \pm 90	144 \pm 149 9-541	<0.001
Hashimoto	4	14.9 \pm 8.0	54 \pm 57 6-111	
Diffuse non-toxic goitre	9	1.0 \pm 1.5	7.4 \pm 9.1 9-19.7	0.01 $p > 0.001$

*Significance of the difference between serum TSH before and the maximal TSH also after TRH stimulation.

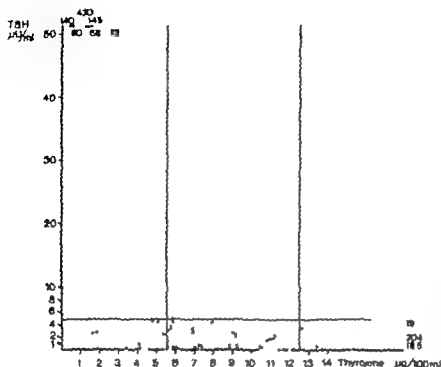


Fig. 1. Relation between serum TSH and serum thyronine in patients with hyperthyroidism during antithyroid treatment (17 samples from 66 patients).

DISCUSSION

Our results regarding normal values of serum TSH are in general agreement with several other studies though our data probably cannot be directly compared to those of other laboratories due to differences in the radioimmunoassay used (12, 14, 19, 26, 27, 30, 36, 38). Like Lemarchand *et al.* (13) and Odell *et al.* (25) no difference in serum TSH between the sexes could be demonstrated. Serum TSH was found to increase with age though significantly only in males. These findings are in accordance with previous studies by Lemarchand-Béraud and Van notti (24) whereas other investigators have found no age-dependent changes in serum TSH levels (34).

In studying the response of serum TSH to TRH different doses of TRH have been used (10, 17, 20, 29, 31, 37). Most authors agree that little diagnostic information is gained by increasing the dose of TRH above 200 µg i.v. and in the present study this dose has been used as a standard procedure. The maximum rise in serum TSH after stimulation with TRH in most cases occurred 20 min following TRH. This finding is in agreement with most authors (1, 11, 14, 18). We found that following serum TSH for more than 60 min after TRH injection no further information was ob-

tained in these disorders. In 2 of the controls a significant increase in serum TSH after TRH stimulation could not be demonstrated. We have no explanation for this lack of response but similar results are described by others using a standard TRH stimulation (1, 5). We have been unable to confirm the results of Jensen and Weeke (16) who found a high positive correlation between baseline TSH and the response to TRH. The reproducibility of the TRH test in the recent study was in reasonable agreement with other studies (10, 34). We could not demonstrate any sex dependent difference in the response to TRH which accords with Snyder and Utiger (34) but is in contrast to Ornston *et al.* (28). In spite of increasing serum TSH the response to TRH diminished significantly with increasing age in males, while no age-dependent alterations could be demonstrated in females. Our finding of a sex difference in age-dependent TSH response to TRH is in accordance with Snyder and Utiger (34, 35).

In our studies of patients with thyroid diseases we found a significant difference in serum TSH between the control group and the group of patients with untreated hyperthyroidism but the overlap in serum TSH values between the groups was so great that a single determination of serum TSH is of no value in the diagnosis of hyper-

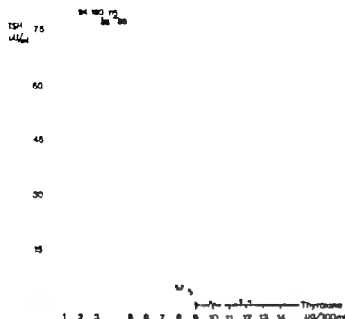


Fig. 2 Relation between serum TSH and serum thyroxine in patients with primary hypothyroidism during treatment with L-thyroxine (79 samples from 34 patients)

thyroidism. The TRH stimulation test, however allowed a good separation between euthyroids and patients with hyperthyroidism, as only one of 56 hyperthyroid patients had a response greater than 2.0 $\mu\text{U/ml}$ and only two of 60 controls responded with less than 2.0 $\mu\text{U/ml}$. Our results concerning the diagnostic value of the TRH test in hyperthyroidism are in accordance with several authors (10 11 18 28). Like in the patients of other investigators (6 10 14 18 28) a considerable elevation both in serum TSH and in the response to TRH was found in all the patients suffering from primary hypothyroidism. In these patients the TRH test offered no further diagnostic information than a single TSH determination. In the 4 patients with Hashimoto thyroiditis serum TSH was moderately elevated and they showed an exaggerated response to TRH although they were clinically euthyroid with serum thyroxine within the normal range. They were probably in a state of preclinical hypothyroidism (21 28). The patients with non-toxic diffuse goitre did not differ from the controls either in serum TSH or in the response to TRH. These findings are in agreement with those of Beckers et al (2).

During antithyroid treatment (Fig. 1) serum TSH was found to be elevated only in half of the patients with subnormal serum thyroxine values. This is probably explained by the finding of normal

serum triiodothyronine levels in these patients (3). In the patients suffering from primary hypothyroidism treated with L-thyroxine (Fig. 2) serum TSH was elevated in 11 cases in which serum thyroxine values were below the normal range. With increasing serum thyroxine values we found in agreement with Cotton et al (4) that serum TSH generally is not normalized until serum thyroxine levels are within the upper part of the normal range. In spite of serum thyroxine levels of the same order it thus appears that serum TSH is higher in patients with treated hypothyroidism than in patients during antithyroid treatment. The lower level of serum TSH in patients during antithyroid treatment might be explained by supposing that these patients have relatively higher serum level of triiodothyronine than those with hypothyroidism treated with L-thyroxine (22).

It can be concluded that a single serum TSH estimation seems to be sufficient in the diagnosis of primary hyperthyroidism. To avoid undertreatment of primary hypothyroidism, estimation of serum TSH also seems to be of value. The finding of elevated values of serum TSH in patients during antithyroid treatment must be considered as sign of overtreatment. In hyperthyroidism a single TSH estimation is of no diagnostic value whereas the TRH stimulation test must be considered a useful diagnostic complement to other thyroid function tests.

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SERUM CALCITONIN IN MEDULLARY THYROID CARCINOMA

Radioimmunoassay Technique and Diagnostic Value

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Abstract. A radioimmunoassay for the determination of human calcitonin in 200 µl aliquots of serum has been developed using rabbit antiserum against extract of an amyloid-producing medullary carcinoma of the thyroid gland (MCT). The assay was specific for human synthetic and native calcitonin and sensitive enough to determine normal and subnormal serum levels. No cross reaction occurred with human or bovine parathyroid hormone. The levels (± 2 S.D.) were 0.70 ± 0.34 ng/ml in 115 control subjects, confirming that calcitonin is a normal serum peptide. High calcitonin levels, > 800 ng/ml, were found before surgery in 1 patient with verified MCT and low levels, < 0.40 ng/ml, after thyroidectomy in 7 of 9 patients thyroidectomized for thyroid tumours other than MCT. Our results show that this calcitonin assay has crucial value in the diagnosis of MCT both primary and metastasizing. It is therefore of value also in the postoperative control of these patients. The levels of calcitonin correlated

well with the clinical estimation of the extent of the disease.

The first radioimmunoassay of human calcitonin (h-CT) was published in 1969 (3) and allowed measurement only of elevated serum levels. In 1970 Tashjian et al. (10) reported an immunoassay which permitted the measurement of normal levels. They applied their assay to three families with medullary carcinoma of the thyroid gland (MCT) (7, 10).

We have developed a sensitive radioimmunoassay of h-CT which measures normal and low levels in untreated serum. The aim of this paper is to present the properties of our assay of h-CT and its clinical usefulness in subjects with MCT known since 1968 to produce calcitonin (10).

MATERIAL AND METHODS

The patient material consisted of 1 patient with amyloid-producing MCT verified by histopathology. Most of them were found by screening for MCT in

families with Sipple syndrome, bilateral MCT combined with pheochromocytomas syndrome inherited as an autosomal dominant trait (9). The other patients were diagnosed by fine needle aspiration biopsy of thyroid biopsies, a routine diagnostic method in our departments (8). A few patients were operated upon only because of raised S-b-CT, in the others, however, there were additional indications for surgery such as nodules in the scintiscan of patient with hereditary Sipple syndrome and/or positive MCT cytology.

The control consisted of 94 healthy adult healthy children, aged 1-19 years, and 9 patients who had previously been subjected to surgically total thyroidectomy for thyroid carcinomas other than MCT.

Isolation and immunoassay standard of h-CT

Synthetic h-CT M from Ciba was used for labelling with N 125-I (Amersham, England). A modification of the chloramine T method of Greenwood and Hunter (4) was used. The synthetic calcitonin was also used as reference standard. The quality of the labelled antigen was tested by chromatoelectrophoresis (2).

Calcitonin-free serum

This was obtained from female 40-year-old patient who had been completely parathyroid- and thyroidectomized for highly differentiated mixed papillary and follicular thyroid carcinoma and primary hyperparathyroidism. She should have no calcitonin-producing cells left and gave a pool of serum. This serum was always added to all tubes of the standard curve in aliquots of 200 µl of 1:10 dilution of her serum, corresponding to the average amount of serum proteins in unknown serum samples.

Production of antibodies to human calcitonin

Antibodies were prepared by injection of six rabbits and five guinea pigs with extracts of human MCT. The glands were freeze-dried, defatted, extracted in 6 M urea, 0.01 M HCl-0.05 M cysteine following the procedure of Aurbach (1) up to the second precipitation step. Initial immunization was obtained by direct injection into spleen and lymph nodes of the extract absorbed on Carbowater E L (Columbia International Ltd.) together with complete Freund's adju-

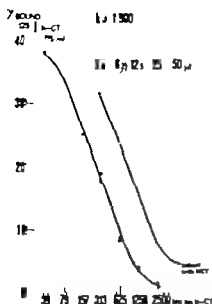


Fig. 1 Linear range of standard curve for serum h-CT using anti-h-CT serum K5 at final dilution of 1:900 and yodheta, h-CT (Ciba) as reference standard. The dilution curve for serum from a patient with MCT is parallel with the standard curve.

ant. The following injection were made subcutaneously. Eight injections of 0.5 mg of a 70% extract per animal during eight months produced antibodies to h-CT in one rabbit and in guinea pigs. The antiserum from rabbit K5 was of sufficient specificity for use in the immunoassay and all data in this paper are based on this antiserum.

Radioimmunoassay

Phosphate buffer 0.05 M pH 7.4 70 μ l Na²²⁴ 0.9 mCi 5–10 μ l h-CT in phosphate buffer 0.1 M pH 7.4 4–5 μ g in 5 μ l chloramine T in phosphate buffer 0.05 M pH 7.4 30 μ g in 70 μ l. Oxidation time was 10 sec terminated by the addition of Na-metabisulphite in phosphate buffer 0.05 M pH 7.4 20 μ g in 70 μ l.

The iodination mixture was transferred to a 1 cm \times 14 cm column of Amberlite CG-400 connected to the acetate size. The column had been equilibrated with and was eluted with sodium acetate buffer 0.05 M pH 5.0. Fractions of 1 ml were collected and the active peak (separated from the damaged ¹²⁵I-h-CT and unreacted Na²²⁴I) usually emerged in tubes 5–10. The labelled antigen was stored at -70°C and could be used for at least one month.

Radioimmunoassay incubation mixture

Standard h-CT 0–5 ng/ml, 0–100 μ l phosphate buffer 0.1 M pH 7.4 0–100 μ l unknown serum 0–200 μ l diluted 10⁻³ serum from the h-CT-free patient 0–200 μ l Trisyl 10³ (Bayer) 400 kU 70 μ l antiserum 1:90, 40 μ l h-CT 100 μ l total volume 400 μ l.

Antiserum K5 was diluted with NaCl 0.14 M containing methiodole, 1:10000. The final dilution of antiserum K5 was 1:900, h-CT 100 and the standard h-CT were diluted in phosphate buffer 0.1 M, with hu-

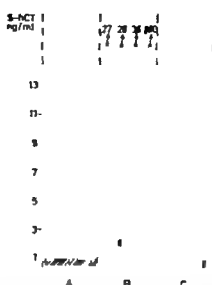


Fig. 2 Serum h-CT levels in 115 healthy control (shaded reference range) (A), before surgery in 1 patients with crurified MCT (B) and in 9 patients thyroid ectomized for thyroid neoplasms other than MCT (C). x = actual measured concentration, o = concentration below detection limit (\sim 0.4–0.4 ng/ml).

man serum albumin (Sigma), 0.25% and bovine sulphate (Sigma), 0.02%. The incubation tubes were sealed and left at 4°C for three days.

Separation of bound and free hormone

Separation of bound and free hormones was accomplished by the dextran-coated charcoal method. Norit A charcoal (Sigma), 8 g, was suspended in 200 ml distilled H₂O and allowed to settle for 30 min. The water was sucked off until 40 ml remained. Dextran (Pharmacia), 500 mg, was added in the charcoal and mixed vigorously. Phosphate buffer 0.1 M, pH 7.4, with human serum albumin 0.01% and bovine sulphate, 0.01% was added up to 200 ml. The suspension was stirred for 1 hour. Of the dextran-coated charcoal 0.5 ml were added to each tube and mixed in Vortex mixer for 3 min. The tubes were immediately centrifuged in cold centrifuge for 6 min at 400 g. The supernatant was sucked off and the dextran-coated charcoal residue containing the free ¹²⁵I-h-CT was counted in a γ -spectrometer for each tube. Control without antiserum K5 was incubated to correct for the incubation damage to ¹²⁵I-h-CT and for non-specific inhibition of the immune reaction by different sera. One control serum with normal and one with known high h-CT levels were included in each set of incubations.

RESULTS

Properties of the radioimmunoassay for human calcitonin in serum

The best anti-h-CT serum came from rabbit K5. When this antiserum was used at a final concentra-

Table I Effects of high concentrations of human and bovine hormones on the assayed h-CT levels

Hormone	Added amount (ng/ml)	Highest value in normal serum (ng/ml)	Assayed levels of h-CT
h-ACTH	1 000	0.1	Not measurable
h-PTH	335	—52	Not measurable
h-PTH	1 000	—	Not measurable
h-STH	50	10	Not measurable

Synthetic peptide (Ciba).

Our local h-PTH preparation purified from adenomas.

Highly purified (1 500 U/mg, Wyeth Co.).

Highly purified, gift by C. A. Demuzell.

tion of 1 900 during 3 days of incubation usually about 25% of added 125 I-h-CT was bound to specific antibodies at zero concentration of calcitonin standard.

Specificity

The anti-h-CT serum hS contained antibodies against h-CT. This is shown by Fig. 1 where a dilution curve of serum from one patient with MCT runs parallel with the linear part of the standard curve. Various concentrations of serum from the patient without functioning thyroid and parathyroid glands did not influence the reaction between synthetic h-CT and its antiserum. A representative standard curve had a linear range between 80 and 1250 pg.

No cross reactions occurred when large amounts of human parathyroid hormone (h-PTH), bovine parathyroid hormone (b-PTH), human adrenocorticotropin (h-ACTH) and human somatotropin (h-STH) were added (Table I).

Reproducibility

The same samples of sera and plasma from one patient with MCT were assayed in 5-6 sets of incubations (Table II). The coefficients of variation were 12% in serum and 18% in plasma.

Table II Variations in h-CT levels in serum and plasma from one and the same MCT patient assayed in different sets of incubations

	Incubations (n)	Individual S-h-CT levels (ng/ml)						Mean	s	Coeff. of variation (%)
Serum	5	3.1	—	—	—	—	—	—	—	—
Plasma	6	3.5	3.1	3.6	—	—	—	—	—	—

Assay

Recovery experiments were done with synthetic h-CT. When 10 ng of the peptide was added to standard normal serum (MCT 90 ng/ml) and when 5 ng was added to serum from patient with MCT (S-h-CT 5.4 ng/ml) the recovery was 170-130%.

Sensitivity and normal range

The sensitivity was adequate enough to permit the measurement of the h-CT concentrations in 10 μ l aliquots of undiluted serum samples. Mean \pm S.D. from 94 adult healthy blood donors was 0.70 ± 0.34 ng/ml with a total observed range of 0.4-1.0 ng/ml (Fig. 1). The levels in children 1-19 years old did not diverge from this range. Usually the linear range of the standard curve would allow the measurement of h-CT down to 0.40 ng/ml, sometimes down to 0.20 ng/ml.

Clinical value

The h-CT levels in peripheral venous blood measured preoperatively in 1 patient with MCT.

We have not seen any patient with MCT histopathology and normal h-CT. False negatives have been found. The h-CT levels were elevated in all patients and ranged from 1.6 up to more than 800 ng/ml (Fig. 1). Total thyroidectomy had been performed in one of these patients 3 years before the examination when he was shown to have multiple lymph node and lung metastases. After removal of these the h-CT level was markedly reduced but still not normal, finding consistent with the presence of pulmonary metastases. Thus, also metastases of MCT produce immunoreactive h-CT.

We have hitherto seen no instance of false positive results. In all patients with high h-CT levels who have been operated upon, the presence of MCT could be verified by histopathology. Generally we found a good correlation between the h-CT level and clinically estimated tumour mass.

Addition of supposedly calcitonin-free serum to normal sera did not influence their normal levels. Low levels were obtained in assays on untreated sera from 7 of 9 patients thyroidectomized for tumours other than MCT.

DISCUSSION

The present work describes the properties of a radioimmunoassay for h-CT and some of its clinical applications.

The most important properties of our antiserum against h-CT are its high specificity and sensitivity. A prerequisite for the use of the assay in studies of calcium metabolism should be the absence of cross reaction for parathyroid hormone. No such cross reaction was observed over a wide range of concentrations of parathyroid or pituitary hormones.

The major drawback of the first radioimmunoassay for h-CT was the low sensitivity permitting only high levels to be measured (3). Our h-CT assay is sensitive enough to determine high normal and usually low levels of h-CT. We found no difference in serum levels of h-CT between healthy children and adults. Our reference values are intermediate between the lower levels 0.02–0.40 ng/ml of the radioimmunoassay technique of Tashjian et al. (10) and the higher levels 0.17–2.5 MRC mU/ml equivalent to 0.6–1.7 ng/ml of the bioassay of Gudmundsson et al. (5).

The value of a laboratory method depends not only on the accuracy and precision of the technique but to an equal extent also on its sensitivity and specificity as a diagnostic method in the clinical situation. In this investigation there was an absolute correlation between the presence of histologically verified MCT diagnosed in the conventional manner and high h-CT levels, i.e. no false negative results were obtained. Nor could we register any false positives. Therefore we conclude. A raised serum h-CT value (≥ 1.5 ng/ml) is a sensitive and specific indication of the presence of MCT.

Both primary and metastasizing MCT produce calcitonin. This means that, postoperatively, determinations of h-CT can be used to check whether the tumour has been completely removed. A

persisting high level of h-CT after surgery should enforce further examinations for metastases.

At present we do not know the clinical significance of h-CT levels close to the upper normal limit. In these cases the h-CT measurements during a 4-hour calcium infusion are of special interest, as described by Tashjian et al. (10) and Melvin et al. (7). The results of our own calcium infusion tests in these borderline cases from three kindreds with Sipple's syndrome will be published later.

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JAUNDICE IN PREGNANCY

A Follow-up Study of the Series of Women Originally Reported by L. Thorling

II Present Health of the Women

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Abstract. In 1955 the late L. Thorling reported on 71 women with jaundice in pregnancy because of cholestasis of pregnancy or infectious hepatitis. A follow-up of 81 of these women, now 40-60 years of age and living all over Sweden, was performed in 1970. Six women had died and four were unwilling to participate. The study included an interview following a standardized questionnaire, oral cholecystography (unless the anatomical state of the biliary tract was known) and physical and laboratory examinations (44 women). A moderately abnormal bromsulphalein retention not accounted for by known disease was found in 8 women, three of whom had had symptoms compatible with recurrent cholestasis of pregnancy. There were few other indications of liver disease and, in general, the women considered themselves to be in good health. Evidence of earlier (cholecystectomy) or present (abnormal cholecystography) gallbladder disease was found in 31 of the 81 women investigated. The prevalence was higher in women above (67%) than below (47%) 50 years of age. Including the deceased women, the maximal prevalence of gallbladder disease among women with recurrent cholestasis of pregnancy was 57% (16 of 28). Three of the 6 deceased women had had severe kidney disease with azotemia, and 12 surviving women had had symptoms of pyelitis and/or nephrotheliasis. Of the latter urinary tract disease was most common among those with non-recurrent jaundice (8 of 25, 32%).

A previous paper (9) reported on the total number of pregnancies in a series of women originally described by Thorling in 1955 (22) because of jaundice during pregnancy. Summarizing the course of all pregnancies, it appeared that the women had suffered from several entities of disorders, the major ones being cholestasis of pregnancy and infectious hepatitis.

Most of the women in the Thorling study were reexamined by us in 1970, i.e. 15 years after the original report. The aim of this follow-up was to

study whether the women suffered from any late sequelae of the episode(s) of impaired liver function. Another object was to investigate whether the women at this time manifested diseases that may have contributed to the development of jaundice during pregnancy. The most important finding is a high prevalence of gallbladder disease among the women with a history of cholestasis of pregnancy.

MATERIAL AND METHODS

Of the 71 women reported by Thorling (22), 61 took part in the present study. Six women had died and four could not be traced or were unwilling to participate.

On the basis of information obtained at interviews, from the Thorling report and from available hospital records, the women were separated into three groups with regard to the recurrence of symptoms (jaundice and itching) (9). In this context, itching alone was considered equivalent to itching and jaundice as sign of cholestasis (1).

Group 1 consisted of 26 women with jaundice and/or itching during more than one pregnancy. Six of them had their major symptoms during the first trimester (group 1 A) while 16 (group 1 B) had jaundice and/or itching during the later parts of all their pregnancies and 4 (group 1 C) during the later parts of some of them. All the women in group 1 B and all but one in group 1 C showed clinical picture compatible with recurrent cholestasis of pregnancy. Recurrent cholestasis with symptoms both early and late in pregnancy also appeared to be the diagnosis in 3 women in group 1 A.

Group 2 comprised 25 women with jaundice during one of several pregnancies, 10 (group 2 A) had negative and 15 (group 2 B) positive thymol turbidity test during their period of jaundice. While several women in group 2 A had symptoms compatible with cholestasis of pregnancy, all but one in group 2 B were originally diagnosed as hepatitis.

The remaining 10 women (group 3) bore

Table 1 Age, cause of death and autopsy findings of the deceased women

Group	Case no. in Thorling report	Age at death (y)	Cause of death	Additional information from autopsy
Recurrent cases				
1 A	31	52	Bronchopneumonia	Chronic pyelonephritis, no uraemia, biliary tract normal
1 B or 1 C	39	43	Mammary carcinoma	Not performed
	41	48	Cerebral haemorrhage	Polycystic kidney disease with uraemia, cholecystectomy
	44	45	Uraemia	Nephropathy, cholelithiasis, steatosis of the liver
Non-recurrent case	69	51	Subarachnoid haemorrhage	Not performed
B	18	31	Accidental	Internal organs normal

child each except one woman, whose only pregnancy ended in abortion. Details of the course of pregnancies have been published elsewhere (9).

All 61 women were interviewed following a standardized questionnaire. Information was also obtained from hospital records. Although they were living all over Sweden it was possible to accomplish physical and laboratory examination in 44, the missing ones (indicated in Fig. 1 as BSP retention unknown) were distributed almost uniformly between the groups. Venous blood samples were analysed for Hb, ESR, alkaline phosphatases (AP), SGPT, bilirubin, creatinine and serum protein (paper electrophoresis). Urine samples were tested for the presence of glucose and protein. Bromsulphalein (BSP) retention tests were performed by measuring the dye in blood samples taken every 5 min for 50 min after the injection of 5 mg/kg b.wt. The concentration of the dye was plotted versus time in order to check that the slopes decreased in a semilogarithmic way.

Women were examined with oral cholecystography unless the anatomical state of the biliary tract was already known. A previous negative cholecystography was accepted if performed less than 5 years before the present study.

The deceased women were subgrouped as described above on the basis of data given in the Thorling report and in hospital records. Additional information was obtained from autopsy reports and death certificates.

RESULTS

Deceased women

Five of the six deceased women had had jaundice and/or itching during several pregnancies (Table 1). One woman (no. 31 in the Thorling report) had been jaundiced in the first trimester of all her pregnancies and she also had had itching during the last trimester in a way similar to three of the women in group 1 A. The cause of death was pyelonephritis associated with uraemia. Severe kid-

ney disease was also found in two other women with recurrent symptoms. In one woman (no. 44), postmortem changes were too advanced to allow a definite diagnosis and the other one (no. 41) suffered from polycystic kidney disease with uraemia. One woman was cholecystectomized and the other one had cholecystolithiasis. The liver and kidneys showed no abnormality in the woman with non-recurrent jaundice (group 2 B) who had died accidentally.

Surviving women

The mean interval between last pregnancy with itching or jaundice and the follow-up varied from 10 to 20 years in the different groups (Table II). In 7 women it was less than 5 years and in 6 more than 20. The mean age at the follow-up was 43-49 years (three below 40 and one above 60).

The histories

Most of the women had been admitted to hospital on one or several occasions for reasons other than complications of pregnancy or childbirth. Apart from biliary and renal tract disease (see below), the most common causes of admission were comparatively simple disorders requiring surgical or gynaecological therapy.

Severe disease had occurred in 5 women. One (no. 48 group 1 B) started to show skin manifestations of systemic lupus erythematosus (SLE) 6 years after her last pregnancy. At the time of the follow-up she had cutaneous, cardiac and hepatic manifestations of this disease and was continuously treated with glucocorticoid hormones. Surgery for mammary carcinoma had been performed in one woman (no. 65 group 2 A) 15 years before the re-examination, still with no evidence of metastases.

Table II. Age at the follow-up study and time between the last pregnancy with itching and/or jaundice and the follow-up study (period of observation) (mean and range)

Group	No of women	Age (y)	Period of observation (y)
Recurrent cases			
1A	6	44.7 (37-50)	16.9 (9-20)
1B	16	47.8 (40-57)	14.4 (2-21)
1C	4	43.0 (35-49)	10.0 (3-15)
Non-recurrent cases			
2A	10	48.5 (39-61)	18.2 (11-20)
2B	15	49.1 (47-55)	20.8 (16-22)
Out pregnancy	10	47.7 (43-58)	18.3 (16-27)

Severe metabolic disease characterized by hyperlipoproteinaemia type V (5) and diabetes mellitus developed in one woman (no. 13 group 2B) more than 10 years after the pregnancy complicated by jaundice. A liver specimen showed marked steatosis; she was treated with insulin. Two women in group 3 suffered from rheumatoid arthritis (no. 21) and congestive heart disease (no. 47). The former received continuous treatment with salicylic acid preparations and the latter was prescribed digoxin and furosemide.

Two women in group 1B had had a febrile disease associated with jaundice during childhood. One woman in group 1B who had suffered from infectious hepatitis during pregnancy had a relapse of this disease (diagnosis confirmed by liver biopsy) 7 years later. She had been well since.

Apart from nos. 48 (SLE), 13 (diabetes mellitus) and 47 (congestive heart disease) no woman who was found to have abnormal laboratory tests was on continuous drug therapy or showed signs of acute infection at the time of the study. None of the women admitted addiction to drugs or alcohol.

Liver disease

The physical examinations revealed a low incidence of abnormal findings compatible with liver disease. Palmar erythema was observed in two women (no. 27 group 1A and no. 26 group 1B). The liver was palpable at the right costal arcus in one woman (no. 52, group 1C) and 1.5 cm below that border in another (no. 8 group 2B). Serum bilirubin, in conjugated and unconjugated, was normal in all women investigated as was SGPT except for

those two suffering from SLE and rheumatoid arthritis respectively. Normal AP values were recorded for all except for the woman with SLE. Hb was more than 12 g/100 ml in all but one woman with kidney disease (see below). Two women had an ESR of more than 70 mm/h; of them also had an increased BSP retention.

Serum protein electrophoresis showed no γ -globulins in 11 women. Four of them also had pathological BSP retention test (see below). Among the remainder the highest γ -globulin value (6 g/100 ml) was encountered in the woman with rheumatoid arthritis. In the others (4 from group 1B, 1 each from groups 2A and 3) the γ -globulins were only moderately increased (1.7-2.0 g/100 ml, normal value ≤ 1.6).

BSP retention at 45 min was more than 5% of the 5 min value in 13 women (Fig. 1, Table III). The three women in group 1A with abnormal BSP retention tests (11-17%) were those who had had symptoms (jaundice and/or itching) only in the first trimester of all their pregnancies. In recent years they had often had slight to moderate epigastric pain. Except for the periods of jaundice during pregnancy there was no history of cholestasis as evidenced by acholic faeces or dark-coloured urine. The γ -globulins were increased in two of the women, one of whom also had palmar erythema. Cholecystography was normal in two of them and showed cholecystolithiasis in the third. It was not permitted a liver biopsy. Serum electrophoresis, serum bilirubin, SGPT and AP were checked again in these women in Jan. 1974 with unchanged results.

Apart from the woman with SLE, three women in group 1B had a somewhat elevated BSP

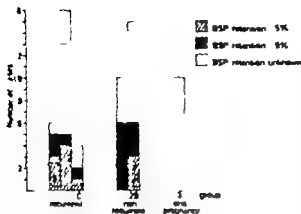


Fig. 1 Outcome of the BSP retention test in relation to itching and jaundice in pregnancy

non. Two of them complained of occasional epigastric pains. Liver biopsy specimens obtained from two of these women were normal as evidenced by light microscopy. A slightly abnormal BSP retention test (9%) and a small elevation of the β -globulins were found in one woman in group 1 C (no. 52). She considered herself to be in good health.

The BSP retention test was performed in 19 of the 25 women with non-recurrent jaundice in pregnancy. Three women in group 2 B showed abnormal values. One was the woman (no. 13) with diabetes mellitus, hyperlipoproteinaemia and stenosis of the liver. The second (no. 9) had cholecystolithiasis and moderately elevated γ -globulins. The third suffered from epigastric pains but had no other signs of liver or biliary tract disease. Two women in group 3 had pathological BSP retention tests. Both were obese and one also suffered from congestive heart disease.

Biliary tract disease

Oral cholecystography was performed in all but 4 of the non-cholecystectomized women (Fig. 2). The incidence of present (cholecystolithiasis, cholecystitis) or previous (cholecystectomy) gallbladder disease was 53% (31 of 61 women). The prevalence was 65% (13 of 20 women) in groups 1 B and 1 C. Gallbladder disease was more common among the women above (67%) than below (47%) 50 years of age. More than 10% overweight was encountered in 11 women. In the major groups there were no

Table III Details about the women with pathological BSP retention tests

group	Case no. in Thorning report	BSP (%)	Electrophoretic pattern	State of biliary system	Liver biopsy	Comments
1 A	3	17	Normal	Normal cholecystography	-	
	77	11	γ -globulin 1.8	Normal cholecystography	-	Palmar erythema
	3	12	γ -globulin 2.0	Cholecystolithiasis	-	
1 B	37	9	Normal	Normal cholecystography	-	
	48	6	γ -globulin 3.27	Normal cholecystography	Normal	SLE
	34	8	Normal	Normal cholecystography	Normal	
	61	8	Normal	Normal cholecystography	Normal	
1 C	52	9	β -globulin 1.6	-	-	Hep. palpable at arcus
2 B	9	15	γ -globulin 1.85	Cholecystolithiasis	-	
	13	19	α_2 -globulin 1.3 β -globulin 1.7	Cholecystectomized	Stenosis of the liver	Diabetes, hyperlipidaemia, Xanthelasma
	22	9	Normal	Normal cholecystography	Normal	
3	7	7	Normal	Normal cholecystography	-	Obesity
	47	8	Normal	Cholecystolithiasis	Normal	Congestive heart disease, obesity

Normal range: BSP <5% α_1 -glob. 0.3-0.7 g% β -glob. 0.5-1.2 g% γ -glob. 0.7-1.6 g%

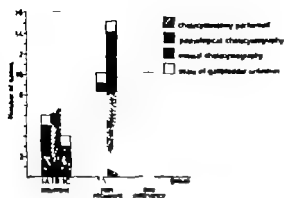


Fig. 2 Prevalence of gallbladder disease in relation to itching and jaundice in pregnancy

significant differences between women with or without present or previous gallbladder disease that could be correlated to age, parity or body weight (Table IV).

Eighteen women had been cholecystectomized. Most of them (13 women) had had the operation after their last pregnancy complicated by itching and/or jaundice (median 8 years, range 0-23). Four women in groups 1 B and 1 C had been cholecystectomized in the interval between complicated pregnancies. One woman in group 2 B had been operated upon one year before her pregnancy associated with jaundice (diagnosed as in-

fectious hepatitis). All gallstones were of the mixed type.

Oral cholecystography showed gallstones in 11 women, 1 my bile in one and a non-functioning gallbladder in another, i.e. findings compatible with chronic cholecystitis (3). X-ray examination had been performed in all 13 women after the last pregnancy with itching or jaundice.

Thorling reported on the result of cholecystography in 17 women performed after a pregnancy with jaundice. Of 17 women with a normal cholecystography at that time 5 have since developed gallstones.

Urinary tract disease

Urinary tract disease necessitating medical care occurred in 3 women in group 1 B, 4 in group 1 A, 4 in group 2 B and 1 in group 3 (Table V). Six women (nos. 42, 48, 9, 11, 22, 67) had been without symptoms for several years. Of the remaining 6 women, 5 had had recurrent infections of the urinary tract for 20-30 years. Three of them continuously treated with antibiotic or chemotherapeutic drugs, were without symptoms while the other two had experienced symptoms during the year preceding this study. The sixth woman was found to have hypertension at the follow-up and the subsequent investigation revealed roentgeno-

Table IV. Prevalence of gallbladder disease (GBD) in relation to age, parity and body weight (mean and range)

Group	Prevalence of GBD	Age (yr)		No. of full-term pregnancies		Ht wt (% of ideal weight) ^a	
		GBD	No known GBD	GBD	N known GBD	GBD	N known GBD
1 A	2/6	46-5	43-8	2-5	2-5	98	107
		43-90	37-49	2-3	2-4	90-106	87-123
1 B	10/16	48-9	43-8	2-0	2-3	96	90
		42-51	40-49	0-4	2-3	83-114	84-87
1 C	3/4	41-7	47	3-7	3	102	91
		35-49		3-4		95-105	
A	6/10	48-2	49-5	3-5	3-0	98	100
		42-61	48-51	2-7	2-4	81-116	92-108
2 B	8/15	49-2	49-1	6	2-6	97	108
		42-55	45-52	2-3	1-4	82-112	81-133
3	2/10	52-5	46-5	1-0	0-9	147	107
		47-58	44-52	1	0-1	138-160	97-132
Total	31/61	48.3	46.8	2.5	1.1	100	102

^aDiagnosis obtained by oral cholecystography or operation; no. of women with GBD/no. of women in the group.

Calculated as $\frac{\text{weight (kg)}}{\text{length (cm)}^2 - 100} \times 100$.

Table V Women with present or previous evidence of urinary tract disease

Group	Case no. in Thorling report	History	Present condition	Urinalysis
1 B	36	Recurrent pyelitis and renal stones	No symptoms	Proteinuria
	42	One attack of pyelitis	No symptoms	Not performed
	48	Two attacks of pyelitis	SLE	Normal
2 A	34	Recurrent pyelitis and renal stones	Long-term nitrofurantoin therapy	Normal
	40	One attack of pyelitis	Hypertension	Normal
			One kidney smaller than the other on X-ray	
	43	One attack of pyelitis Recurrent cystitis	One kidney slightly smaller than the other on X-ray	Normal
	66	Recurrent pyelitis	Ampicillin therapy for bacteriuria	Normal
B	4	Recurrent cystitis	Long-term sulphonamide therapy	Proteinuria
	9	Renal stones	No symptoms	Normal
	11	Three attacks of pyelitis	No symptoms	Normal
	22	One attack of pyelitis	No symptoms	Normal
3	67	Renal stones	No symptoms	Proteinuria

logical changes compatible with chronic pyelonephritis. Jaundice and/or itching during pregnancy appeared in association with an attack of pyelitis in 4 women (nos 42 and 48 in group 1 B, nos 40 and 43 in group A). Serum creatinine was normal in all investigated women, as were urine analyses in all but 3 with known urinary tract disease.

n to oral contraceptive

61 women: 11 had tried oral contraceptives (group 1 A, 4 in group 1 B, 1 in group 1 C, 1 in group 2 A, 1 in group B and 1 in group 3). The period of treatment varied from 3 days to 18 months (median 4 months). Eight women stopped taking the pills because of side-effects, the most common one being a feeling of sickness (5 women). One woman only (group 1 B) got itching, which developed on the third day of medication.

DISCUSSION

In his report on the present women, Thorling distinguished three groups of disorders, i.e. jaundice in early pregnancy, jaundice in late pregnancy and infectious hepatitis (22). The main basis for this distinction was the histones and the outcome of the thymol turbidity tests. Summarizing the course of all the pregnancies in these women, it

appeared that the Thorling diagnoses represented heterogeneous entities of disorders. Other pregnancies not included in the Thorling study were either not complicated by symptoms of liver disease or were complicated by pruritus and/or jaundice.

Upon reclassification of the women according to the principles outlined above and described in detail in a previous paper (9), the disorder represented by the women in group 1 B seemed to be homogeneous. This condition reappearing during the later parts of all pregnancies was entirely compatible with recurrent cholestasis of pregnancy. Half of the women had a strong familial predisposition to develop these symptoms during pregnancy. Except for one woman, the symptomatology presented by the women in group 1 C (partial recurrence of symptoms) was similar to that of the women in group 1 B.

The pregnancies of the women in groups 2 A ("non-hepatitis") and B ("hepatitis") were somewhat heterogeneous with regard to type and course of symptoms. This may be because the thymol turbidity test is occasionally abnormal in normal pregnancies (6, 11). It has also been suggested that the incidence of positive tests is lower than otherwise when hepatitis is associated with pregnancy (74). Thus the possibility that some of the women in group A and not all of those in

Table VI. Prevalence of gallbladder disease (GBD) in the present series and in two autopsy series from Malmö General Hospital

Figures within parentheses denote percentages

Age (y)	Present series		Autopsy series reported by Sierby		Autopsy series reported by Lindström	
	Total no.	GBD (no.)	Total no.	GBD (no.)	Total no.	GBD (no.)
10-39	3	2	19	2 (11)	1	4 (33)
40-49	40	17 (43)	61	11 (26)	40	6 (15)
50-59	17	11 (65)	105	46 (44)	76	30 (39)
60-69	1	1	126	69 (55)	180	95 (53)
70-79	-	-	19*	134 (70)	96	186 (63)
80-89	-	-	175	108 (62)	376	237 (63)
90-99	-	-	13	12 (92)	77	48 (62)

The study (20) covered 64% of all women who lived in the defined area (the city of Malmö) and who died during April 1961 - March 1962.

The series (14) included 88% of all persons above 20 years of age who died in the same district in 1969.

group 2 B suffered from hepatitis has to be kept in mind.

In view of the long interval between the Thorling report and the present study this follow-up offered an unique opportunity to evaluate the possible existence of late sequelae of jaundice in pregnancy in one of the largest and earliest series of women with this complication. There was some difficulty in persuading the women who now live all over Sweden to participate in both the interview and the physical and chemical examinations. But, since the drop-out rate is similar in all subgroups the fact that only 44 of 61 women fulfilled all parts of the study does not seem to invalidate the general conclusions.

The histories provided no indication that the women in the large groups (1 B, 2 A and 2 B) suffered from severe liver disease and this impression was supported by the findings of the physical and laboratory investigations. Excluding the women with SLE the BSP retention test was slightly elevated in only 3 of the women in group 1 B (recurrent cholestasis of pregnancy). The liver biopsy specimens obtained from two of these women were morphologically normal as evidenced by light microscopy. Slightly abnormal BSP retention tests were encountered in only two of the women in group 1 B (the woman with diabetes mellitus and hyperlipoproteinemia not included) who probably had had infectious hepatitis. One of these women had cholecystolithiasis and elevated γ -glob-

ulins. For other women in this group had normal BSP retention tests but showed some elevation of the serum γ -globulins. A similar finding was also recorded in one woman in group 2 A.

Although some of the women mentioned above may have some degree of liver disease the results indicate an excellent prognosis with regard to liver function not only after recurrent cholestasis of pregnancy but also following infectious hepatitis during pregnancy. Other studies generally covering much shorter periods of observation, support this conclusion (8, 10, 11, 12). Residual liver damage following recurrent cholestasis of pregnancy as evidenced by impaired BSP retention tests has been reported for a few women (4, 11). Elevated serum γ -globulins have been observed in follow-up studies of subjects with infectious hepatitis (8) including women with hepatitis in pregnancy (1).

Three women of particular interest are those in group 1 A (nos 3, 77 and 32) with jaundice and/or itching during the first trimester of all pregnancies ending in childbirth ($n=6$). The symptoms developed in association with hyperemesis gravidarum, and the women had been exposed to viral hepatitis before their first pregnancy. At the time of the follow-up they all experienced epigastric pain and showed evidence of liver dysfunction. The women were unwilling to participate in further investigations and the nature of their disease remains obscure.

For one part of Sweden the prevalence of gallbladder disease in the general population may be estimated. Most autopsies in the city of Malmö (population 260 000) in the south of Sweden are carried out in one hospital. For women 40-49 and 50-59 years of age the prevalence of gallbladder disease in two autopsy series from this hospital (14%) averaged 22 and 47% respectively (Table VI). The corresponding values in the present investigation were considerably higher—43 and 65% in the age groups 40-49 and 50-59 years respectively. In the autopsy series the prevalence exceeded 60% only in women above 70 years of age.

Altogether 13 (65%) of the 20 women in groups I, II and I C (recurrent cholestasis of pregnancy) suffered from gallbladder disease or had been cholecystectomized because of cholelithiasis. Including the 3 women in group I A (nos. 29, 30 and 33) and the deceased women (nos. 31, 43, 48, 45 and 51) who probably also had recurrent cholestasis of pregnancy the overall minimal prevalence of gallbladder disease in this disorder at the time of the follow-up was 57% (16 of 28 women).

The prevalence of gallbladder disease is known to increase with age and to be higher in women than in men. Although it is not possible to obtain a figure for the overall prevalence of such disease it appears to be higher in Sweden than in several other countries (11). The figures recorded for the present women with recurrent cholestasis are extraordinarily high and not far from those reported for female Pima Indians in Arizona (19). These women are reported to have a reduced pool size of the two primary bile acids cholic acid and chenodeoxycholic acid (23) which may or may not be of pathogenetic importance for the formation of gallstones.

Biliary tract disease has been associated with overweight and with parity. However in the series of Swedish women reported by van der Linden (13) only a weak correlation was observed between gallbladder disease and obesity. Comparisons between the women with and without gallbladder disease in the present study did not reveal any differences in body weight. In the Framingham study (7) the prevalence of gallstone disease was found to be higher in parous than in non-parous women. Among the women in the large groups of the present study parity was about the same whether they showed gallbladder disease or not. It seems likely that factors other than those mentioned

above have contributed to the high prevalence of this disorder.

A number of studies in recent years have demonstrated a disturbed metabolism of steroid hormones in pregnant women with cholestasis of pregnancy (1). The hypothesis that steroid hormone metabolites contribute to the development of impaired bile secretion in this condition gained support from the observation that some women with previous cholestasis of pregnancy react with cholestasis during treatment with oral contraceptives. It has been speculated that these patients have some genetic predisposition to impaired bile secretion not only during pregnancy but also intermittently in the non-pregnant state as a result of a cyclic variation of hormones (1). Such a failure may cause an impaired micelle formation and consequently contribute to the formation of gallstones. This hypothesis is supported by the present study insofar as several of the women with recurrent cholestasis of pregnancy have a strong familial predisposition to develop cholestasis during pregnancy (9). One of the 4 women in group I B who were subsequently on oral contraceptives developed itching during therapy.

A high incidence of gallbladder disease was also encountered among the women in groups A (16 of 10) and B (8 of 13) who were supposed to have suffered from non-recurrent cholestasis and viral hepatitis. This finding in the latter group raises the question whether these women had pigment stones rather than cholesterol-rich concretions. However there was no evidence of haemolytic anaemia or liver cirrhosis (15) and all stones removed at operation were of the mixed type.

Next to abnormalities of the liver and the biliary tract, evidence of urinary tract disease was the most common finding at the follow-up. Three of the 6 deceased women had had severe kidney disease with uraemia and 17 surviving women had had symptoms of pyelitis and/or nephrolithiasis. While these symptoms seem to have been sporadic in 6 women they had probably been recurrent for many years in one woman in group I B, four in A and one woman in group B. Including two women from group A altogether four women had had an attack of pyelitis during the pregnancy studied by Thorling. Jaundice had appeared in association with attacks. A similar connexion between jaundice

in pregnancy and pyelitis has been observed by others (12, 16, 17, 18).

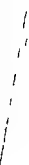
In his original report on the present women Thöring suggested that jaundice during pregnancy may occasionally be precipitated by extrahepatic infections. The present follow-up of the same subjects demonstrates that altogether 8 (32%) of 25 women with non-recurrent jaundice of pregnancy showed or have shown evidence of urinary tract disease (pyelitis, nephrolithiasis or recurrent cystitis). This finding underlines the importance of further investigation into the possible association between jaundice of pregnancy and renal diseases. In contrast to women with recurrent cholestasis of pregnancy there was no conclusive evidence of a familial predisposition to develop jaundice and/or pyelitis in women with non-recurrent symptoms (9).

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REGIONAL CEREBRAL BLOOD FLOW CEREBRAL METABOLIC RATE OF OXYGEN AND CEREBROSPINAL FLUID ACID-BASE VARIABLES IN PATIENTS WITH ACUTE MENINGITIS AND WITH ACUTE ENCEPHALITIS

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Abstract. Regional cerebral blood flow (rCBF), cerebral metabolic rate of oxygen (CMRO₂), jugular venous P_{aO₂} and cerebrospinal fluid (CSF) acid-base variables have been studied in 11 patients with meningococcal meningitis, 11 with pneumococcal meningitis and 11 with encephalitis. The ¹³³Xenon intra-arterial injection method was used for the rCBF measurements. The patients were hyperventilating spontaneously. The studies are performed during general anesthesia and artificial ventilation at P_{aCO₂} adjusted to the level measured during spontaneous respiration prior to the anesthesia. Average hemispheric CBF was normal and CMRO₂ slightly reduced in patients with meningococcal meningitis, whereas CBF and CMRO₂ were markedly reduced in pneumococcal meningitis and encephalitis. Jugular venous P_{aO₂} was increased ("luxury perfusion") in meningococcal and pneumococcal meningitis but normal in encephalitis. CSF lactate acid was increased and CSF bicarbonate decreased in all meningitis patients and some in encephalitis. Functional tests of the rCBF were performed by changes of arterial BP and P_{aO₂}. Autoregulation (rCBF is normally independent of the BP within wide limits) was frequently impaired, whereas the response to P_{aO₂} changes was preserved in most cases (rCBF normally increases with increased P_{aO₂}). Focal rCBF abnormalities as disclosed in the control condition during the functional tests were infrequent in meningitis but frequent in encephalitis. The results suggest that the brain of these patients is vulnerable to factors such as hyperventilation, hypercapnia and hypoxia. A more deliberate use of sedatives combined with artificial hyperventilation, advocated. Epileptic seizures should be counteracted by all available means.

In acute meningitis and encephalitis global and sometimes focal cerebral symptoms will be present due to more or less widespread inflammatory and edematous changes in the tissue. It was the aim of the present study to investigate some of the

pathophysiological mechanisms involved in these processes.

MATERIAL AND METHODS

Sixteen patients above 15 years of age entered the study. Five had acute meningococcal meningitis, five pneumococcal meningitis, and six acute encephalitis.

The patients with meningococcal and pneumococcal meningitis had typical moderate to severe symptoms to 5 days duration (average 3.5 days) and were studied within the first 4 hours after the diagnosis was established and antibiotic treatment was started. One patient with severe meningococcal meningitis was however first studied after 48 hours. At the time of the study four patients with meningococcal infection were comatose and agitated, whereas one was awake but lethargic. Two patients with pneumococcal infection were comatose and agitated. The remaining three were stuporous. All 11 patients with meningococcal infection recovered. Two of five patients with pneumococcal infection recovered, two survived but with neurological deficit, and one died due to accumulation of secretions in the airways.

The patients with acute encephalitis were studied within the first 1-2 weeks of the disease. The etiology was unknown, although herpes simplex was suspected in two cases based on increasing antibody titer in blood. No brain biopsy was performed. At the time of the study two of the six patients with encephalitis had rather moderate symptoms, lethargy, agitation, confusion and headache. The other four patients had severe symptoms, more or less pronounced coma and in one case marked rigidity. Two had decerebrate posturing and three had seizures. Artificial respiration was instituted in three cases. The same pattern of abnormalities of the recorded variables as found in the patients with moderate and with severe encephalitis. All six patients with encephalitis recovered after 3-10 weeks of hospitalization.

The investigations were carried out under general anesthesia and artificial ventilation using time-cycled

Table 1 CBF, CMRO₂, jugular P_iO₂ and CSF findings in anesthetized patients with meningitis and encephalitis

	No. of pts.		Age (y)	P _a O ₂ (mmHg)	MABP (mmHg)	Rectal temp. (°C)	Control CBF _{mean} (ml/100 g/ min)	Control CBF _{max} (ml/100 g/ min)
Meningococcal meningitis	5	Mean	20	3	94	38.6	65	66
		S.D.		5	3	0.8	18	10
		Range	15-35	4-38	90-97	38.1-39.8	43-85	45-69
Pneumococcal meningitis	5	Mean	54	77	86	38.4	35	33*
		S.D.		6	16	0.7	8	5
		Range	40-63	19-33	76-113	37.4-39.0	28-49	29-42
Encephalitis	11	Mean	79	31	81	37.9	79	31
		S.D.		7	11	0.7	9	10
		Range	17-98	19-38	66-91	37.0-39.1	17-41	21-46
Normal values in awake man		Mean		40	90	37.0	64	60
		S.D.		1			9	5

The P_aO₂ values in this Table represent those measured during spontaneous respiration before the measurements. The average values corresponding to the CBF measurements which were performed during artificial ventilation deviated less than 1 mmHg from the average P_aO₂ values given in the Table.

respirator (Lundvall). The anesthesia was induced with about 200 mg pentobarbital and about 20 mg d-tubocurarine chloride. It was maintained with oxygen and nitrous oxide at the ratio 1:2 and continuous relaxation was secured with repeated small doses of d-tubocurarine chloride. The ventilation was adjusted in order to maintain the arterial carbon dioxide tension (P_aCO₂) close to that during spontaneous ventilation prior to the anesthesia. The P_aCO₂ levels during artificial ventilation deviated from those of spontaneous respiration by on an average -0.5 mmHg with a S.D. of 0.5 mmHg. No major differences were found between the three groups of patients.

Blood samples were drawn from two small polyethylene catheters (i.d. 1 mm) placed into the bulb of one of the internal jugular veins and into one of the internal carotid arteries (Seldinger technique). Most often the venous and the arterial catheters were placed on the same side.

Cerebrospinal fluid (CSF) was collected aseptically from the cisterna magna after suboccipital puncture.

The regional cerebral blood flow (rCBF) was measured using the ¹³³Xenon intra-arterial injection method (9). Briefly this method utilizes rapid injection of ¹³³Xenon dissolved in saline into the internal carotid artery via the polyethylene catheter and external registration of the ¹³³Xenon clearance curve from the multiple areas of the hemisphere using small scintillation detectors. In the present study 14 scintillation detectors were used. Each crystal (NaI(Tl)) in the scintillation detectors measured 11.5 mm in diameter and 5 mm in thickness. They were collimated by 43 mm long cylindrical lead tubes with an i.d. of 1 mm. The rCBF was calculated from the initial part of the clear-

ance curves (rCBF_{initial}) as well as from the clearance curves followed for 10 min (rCBF₁₀). The rCBF₁₀ and the arteriovenous oxygen difference ((a-v) O₂) were used for the calculation of the cerebral metabolic rate of oxygen (CMRO₂): CMRO₂ = CBF₁₀ × (a-v) O₂.

P_aO₂, P_aCO₂ and pH were measured using conventional electrodes (Radiometer) and corrected for the patient's abnormal body temperature (7-14). Oxygen content in arterial and venous blood was calculated from the corresponding values of P_aO₂, P_aCO₂, pH, Hb, and body temperature and used for the calculation of (a-v) O₂. Lactic acid was determined by an enzymatic method (1). Bicarbonate in the CSF was measured by titration using an automatic equipment (Radiometer). Mean arterial blood pressure (MABP) was measured in the internal carotid artery with an electric sphygmometer (in a few patients on the arm by auscultation). The CSF pressure was recorded using the manometer of CSF as a tube.

A control CBF measurement (i.e. the P_aO₂ close to that during spontaneous respiration) was followed by study during moderate hypertension induced by infusion of angiotensin (1 mg/l). Then followed in several patients a second control study and thereafter a study during induced hypo- or hypercapnia. The P_aCO₂ clamp was 5-15 mmHg. Most patients with P_aCO₂ levels below 25 mmHg during spontaneous respiration were exposed to an increase, and most of those with P_aCO₂ above 25 mmHg to reduction of P_aCO₂. In several patients subsequent final flow study was performed at the changed level of the P_aCO₂ during moderate hypertension induced by a new angiotensin infusion. At the end of the study the catheters were removed. No complications occurred.

In-vivo O_2 (ml/100 g/ min)	CMRO ₂ (ml/100 g/ min)	Jugular PvO ₂ (mmHg)	CSF			Leucocytes/ μ l	Pressure (mm Hg)
			Bicarbonate (mmol/l)	Lactate (mmol/l)			
41	2.3	49	15.5	7.7		514	126
15	0.5	4	3.1	1.8		716	86
16-19	1.7-1.8	44-54	10.3-18.1	6.0-10		1437-18500	190-400
48	1.6	46	16.4	9.8		97	70
11	0.6	6	3.4	1		837	166
13-18	1.1-1.1	40-53	12.4-20.0	3.9-8.4		37-6077	40-280
14	1.7	38	18.4	3		10	130
14	0.3	5	3.1	1.1		13	44
15-23	1.3-1.1	33-47	15.6-1.6	0-1.0		1-33	80-160
56	3.3	38	13.6	1.5		<3	125
	0.5	3	0.9	0.3			

Values from 4 patients, values from 5 patients, values from 3 patients.

RESULTS

Cerebral metabolism, average hemispheric blood flow in control condition, jugular venous P_O₂ and CSF acid-base status

The average values, S.D. and ranges of the monitored variables in the different groups of patients are given in Table 1. Rather marked variations were seen in all groups.

All patients were hyperventilating prior to the average P_aCO₂ being about 30 mmHg.

The average hemispheric blood flow was normal in patients with meningococcal meningitis but reduced by 30-40% in patients with pneumococcal meningitis and with encephalitis.

For the evaluation of CMRO₂ the anaesthesia and the body temperature must be taken into consideration. Based on the other studies of anesthetized patients in our laboratory (1) it can be expected that the anaesthesia used will reduce the CMRO₂ by about 20% (to ≈ 6 ml/100 g/min). A similar effect of the anaesthesia on the CBF may also be expected. The influence of hyperthermia on the CMRO₂ is to some extent uncertain, in contrast to the influence of hypothermia resulting in the well known CMRO₂ reduction. Therefore corrections of the CMRO₂ due to hyperthermia are not made. In the present study the CMRO₂ was reduced in all three groups of patients. The reduction was about 30% in the patients with meningococcal meningitis and was thus not significantly different from what might be expected

result of the anaesthesia. In the patient with pneumococcal meningitis and with encephalitis the reduction was about 50% and thus much more marked than that due to the anaesthesia.

The jugular venous oxygen tension (P_jO₂) was normal in the patients with encephalitis but significantly elevated in those with meningitis. The increase in the jugular PvO₂ indicates that the CBF is higher than that corresponding to the tissue's demand of oxygen—the so-called luxury perfusion syndrome (3).

Studies of the cerebrospinal fluid revealed in patient with meningitis a metabolic CSF acidosis (increased lactate and reduced bicarbonate concentration). Blood lactate concentrations were normal. Patients with encephalitis showed only acidosis was only partially compensated by hyperventilation so that the CSF pH values were still below normal. In some cases the CSF pH values were within the normal range or slightly above normal. Patients with encephalitis showed only minor CSF abnormalities compared with meningitis patients. The presence of leucocytes in the CSF may contribute to the formation of lactic acid (8). This can however not be the only factor since increased CSF lactate concentrations were also seen in patients with encephalitis in whom the CSF pleocytosis was only minimal. The acid-base studies of the cerebrospinal fluid are reported in further details in a subsequent communication (5).

We found significantly negative linear correlation

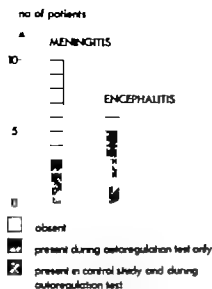


Fig. 1 Focal rCBF abnormalities in patients with bacterial meningitis and with encephalitis.

tion between the CSF pH and log CBF ($p < 0.01$) and between the CSF pH and the jugular PvO_2 ($p < 0.01$). These findings confirm that the brain extracellular fluid pH is a main factor determining CBF (4, 16). In addition the findings support the view that the luxury perfusion syndrome is related to acidosis in the brain (3).

abnormalities of the cerebral blood flow during the control condition

Focal rCBF abnormalities in the form of hyperemic foci were infrequent in meningitis but frequent in encephalitis. Ischemic foci were only seen in one patient with encephalitis who also had a hyperemic focus (Figs. 1 and 2, Table II).

A focus is disclosed by the deviation of the flow values in one or more neighboring channels from the values in the rest of the hemisphere. A marked flow deviation in a few channels or a slight deviation in many adjacent channels and the technical quality of the clearance curves including the counting rate (low in peripheral channels) are important factors which have to be taken into consideration in the evaluation of a focal rCBF abnormality. Thus no simple definition can be given for a focal abnormality. In the present study all focal flow abnormalities encountered in the control condition showed marked deviations of the rCBF of $\geq 5\%$ of more from the rest of the hemisphere.

Foci with less marked abnormalities were not seen.

Regional cerebral blood flow findings during functional tests

In addition to the measurements in the control condition (at a Pa_{CO_2} as close as possible to that during spontaneous respiration) the rCBF was measured during one or more functional tests with arterial BP or Pa_{CO_2} changes. Normally the rCBF is unchanged during changes of the arterial BP within wide limits—so-called autoregulation. Normally rCBF increases when the Pa_{CO_2} is increased (about 4%/mmHg increase of the Pa_{CO_2}).

The rCBF abnormalities encountered are summarized in Table II and consisted of

Control: Pa_{CO_2} 35 mm Hg, MABP 85 mm Hg



Hypertension: Pa_{CO_2} 36 mm Hg, MABP 108 mm Hg



CBF index (ml/100g/min)

Fig. 2 Grossly abnormal regional flow pattern in a patient with encephalitis (no. 19). In the control condition (upper figure) a hyperemic focus is present in the frontal region with flow values marked as numbers (rCBF ml/100 g/min) above those in the rest of the hemisphere and an ischemic focus is present in the parieto-occipital region. Induced hypertension (lower figure) disclosed an impairment of the autoregulation in the peripheral frontoparietal region with an increase of the rCBF and a large frontotemporal area with paradoxical flow decrease.

Table II CBF abnormalities in anesthetized patients with meningitis and encephalitis

Case no	Meningococcal meningitis					Pneumococcal meningitis					Encephalitis					
	2	3	8	13	1	4	11	17	18	22	9	10	11	14	19	20
Focal hyperemia at "normocapnia"	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	-
Focal ischemia at "normocapnia"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Globally impaired autoregulation at "normocapnia"	+	-	+	-	-	+	-	+	+	+	-	-	-	+	-	-
Focally impaired autoregulation at "normocapnia"	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-
Paradox autoregulation	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Restoration of autoregulation at "hypocapnia"														+	-	
Induced loss of autoregulation at "hypercapnia"								+			+	-	+			-
Globally impaired CO ₂ response	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Focally impaired CO ₂ response	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

+ = present - = absent, "normocapnia" refers to PaCO₂ about that during spontaneous respiration before the rCBF study "hypocapnia" to below and "hypercapnia" to above.

1 Globally impaired autoregulation (throughout the whole hemisphere studied) (Fig. 3)

- Focally impaired autoregulation (in circumscribed area of the hemisphere studied) (Figs. 2 and 4)

3 Focal paradoxical or false autoregulation consisting of an abnormal focal rCBF decrease in a region of the hemisphere simultaneously to lost autoregulation with rCBF increase in the other parts of the hemisphere during induced hypertension (10:1) (Fig. 5)

4 Globally impaired response to PaCO₂ changes.

5 Focally impaired response to PaCO₂ change.

In some patient the autoregulation was tested at two different levels of the PaCO₂ and some additional abnormalities were seen

6. Focal restoration of autoregulation during decrease of the PaCO₂

7 Focally induced loss of autoregulation during increase of the PaCO₂

All these types of rCBF abnormalities are similar to those seen in patient with epilepsy or other types of acute cerebral lesions (10:11)

In some of the patients with global impairment of the autoregulation or the response to PaCO₂ changes the impairment was especially pronounced in focal region. In these instances both focal and global loss of autoregulation or of the response to PaCO₂ changes has been indicated in Table II

The autoregulation was considered to be globally impaired if the average rCBF increased more than 10% in response to induced hypertension with a MABP increase of about 30 mmHg (constancy of the PaCO₂ was secured) and in most cases with globally lost autoregulation the average rCBF increased about 20% in response to hypertension. Globally impaired response to PaCO₂ changes (increase or decrease of about 10 mmHg) was seen only in few patients in whom the normal response was nearly completely abolished.

Whereas the global abnormalities can easily be characterized by numerical value it is more difficult to describe the focal abnormalities since among other things, the size of the focus is of importance as discussed above. In the patients

MABP 76mm Hg \rightarrow 106mm Hg
 PaCO₂ 21mm Hg \rightarrow 20mm Hg
 CBF_{total} changes in % in circles



Fig. 3 Globally impaired autoregulation in a patient with pneumococcal meningitis (no. 4).

MABP 66mm Hg \rightarrow 100mm Hg
 PaCO₂ 27mm Hg \rightarrow 26mm Hg
 rCBF_{total} change in % in circles

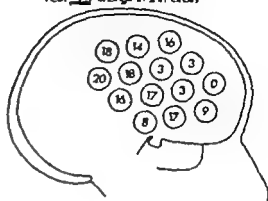


Fig. 4 Focally impaired autoregulation in a patient with encephalitis (no. 10).

with focal loss of autoregulation the focal flow increase during hypertension ranged between 9 and 38%. Focal loss of the response to PaO₂ was only seen in one patient with encephalitis who had grossly abnormal rCBF with marked heterogeneity of the flow responses.

Impairment of autoregulation was a much more common finding than impairment of the response to PaCO₂ changes (Table II). Such a stage with dissociated vasoparalysis has previously been observed outside the focal lesion in patients with intracranial tumors and with cerebral apoplexy. Hypocapnia could restore the autoregulation (13) the present study the dissociated vasoparalysis could in some instances be restored by decreasing the PaO₂ or could be provoked by increasing the PaO₂ (Table II).

Focal abnormalities of the rCBF during the functional tests as also focal abnormalities in the control condition were more frequent among patients with encephalitis than with meningitis (Table II Fig. 1).

DISCUSSION

Shapiro and Eisenberg (15) have used the nitrous oxide inhalation technique to study the CBF and metabolism in five patients with St. Louis encephalitis (4 conscious, 1 comatose). The method measures the average CBF. All their patients had a normal CBF but the CMRO₂ showed variations on both side of the normal and was especially low in the comatose patient in whom the (A) O₂ also was reduced. In this patient luxury perfusion

was thus present. These authors' results differ from those in the present study where CBF and CMRO₂ decreases were characteristic findings. These differences are probably due to differences in the nature of the infectious process in the various forms of encephalitis as the methods used for the CBF measurement both by Shapiro and Eisenberg and ourselves are highly reliable and give comparable results.

In the present study the ¹³³Xenon intra-arterial injection method was used for the flow measurements. This method renders determination of the rCBF possible as each external scintillation detector measures the flow (¹³³Xenon clearance) from a block of cerebral tissue represented by a truncated cone throughout the hemisphere. The results demonstrated that focal rCBF abnormalities were common in encephalitis but uncommon in meningitis. This difference may be related to pathological changes. In encephalitis the inflammatory processes primarily affecting the brain may have a focal and not only a global character as is well known for example in several cases of herpes simplex encephalitis. On the other hand in bacterial meningitis the infectious process is in most cases rather diffuse throughout the subarachnoidal space and may invade the adjacent brain uniformly. Therefore using the ¹³³Xenon intra-arterial injection method one will get a rather uniform flow pattern over the hemisphere although differences may well occur between more superficial structures in the hemisphere (in the truncated cones measured from) and deeper struc-

tures at a greater distance from the subarachnoidal space.

From a pathophysiological point of view the present study indicates that in patients with acute meningitis or encephalitis the brain is highly vulnerable. Factors such as hypertension, hypercapnia and hypoxia may even if moderate provoke an aggravation of the patient's condition.

Impairment of cerebral autoregulation (vasoparalysis) implies that CBF increases when the arterial BP is raised. This means that the arteriolar BP is transmitted to the capillaries, thus increasing the risk of brain edema during hypertension. Episodes of hypertension may well occur during confusion and agitation. It would therefore appear rational to avoid peaks of hypertension by the use of sedative. If depression of respiration cannot be avoided, sedation must be combined with artificial ventilation as discussed in the following.

Hypercapnia leads to an aggravation of an intra-cerebral acidosis. This is because the pH in the brain depends upon the ratio between HCO_3^- and CO_2 and because CO_2 crosses the blood-brain barrier rapidly whereas the passage of HCO_3^- is impeded. CBF is mainly determined by the pH of the brain extracellular fluid. Lowering of pH causes cerebral vasodilation and vice versa (4, 16, 17). Hypercapnia thus increases CBF and may also induce loss of autoregulation. In addition hypercapnia increases the intracranial pressure. In one patient (no. 1) with an intracranial pressure of 400 mm water in the control condition at PaCO_2 of 5 mmHg, the increase of the PaCO_2 to 33 mmHg resulted in a rise of the IABP from 97 to 140 mmHg (Cushing effect?). The PaCO_2 was immediately lowered again. The case illustrates that even moderate PaCO_2 increases to levels well below the normal value of 40 mmHg may cause dangerous effects on the brain. The diseased patient may become exhausted due to continuous vigorous hyperventilation and/or marked agitation. Under such circumstances as well as after deliberate use of sedatives a decreasing PaCO_2 may indicate that respiration should be assisted and PaCO_2 kept at the hypocapnic level shown by the patient before exhaustion.

Hypoxia leads to increased production of lactic acid within the brain. Thus hypoxia also aggravates an intracerebral acidosis and consequently enhances a luxury perfusion with risk of brain edema.

Epileptic seizures frequently accompany acute meningitis and encephalitis. The epileptic seizure in itself hypertension, hypercapnia, hypoxia and increased cerebral metabolism (2, 6). All four factors may be dangerous to the vulnerable brain in these patients. We would therefore propose effective prevention of repetitive seizures. If necessary heavy doses of barbiturates should be combined with artificial ventilation.

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ENZYME ACTIVITIES AND GLYCOGEN CONCENTRATION IN SKELETAL MUSCLE IN ALCOHOLISM

The Effect of Abstinence and Physical Conditioning

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Abstract Muscle metabolism of chronic alcoholics has been studied using muscle biopsy techniques immediately after drinking period, after 6-7 days abstinence and after one month physical conditioning. The activities of CPK, HK, LDH, MDH and SDH were significantly decreased in musculus vastus lateralis for 1 day after an alcoholic debauch. The enzyme activities of the alcoholics, who either had been abstinent for 6-7 days or in addition conditioned for one month, did not differ from those of the controls. The concentration of muscle glycogen was at the same level in both groups, but in bicycle ergometer work of an equal relative intensity the alcoholics used more glycogen than the control subjects. The results suggest that the energy metabolism of skeletal muscle is disturbed in an acute stage of chronic alcoholism as judged from the activities of some key enzymes of energy-yielding metabolic pathways. Enzyme activities are normalized during the first week of abstinence, but the consumption of glycogen in physical work still remains larger than in normal muscles after one month's conditioning due to difference in physical fitness.

It is well known that muscle metabolism of alcoholics is disturbed (7) but direct evidence has been obtained only recently when muscle biopsy techniques and microanalytical methods were adapted for this purpose. In an acute alcoholic myopathy the serum concentrations of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) are increased (7-11). In muscle of acutely intoxicated alcoholics the concentration of LDH 5, which is the predominant LDH isoenzyme in normal human skeletal muscle, is decreased (9). According to Nygren (8) this change is, however, unspecific and may also occur in connection with other acute diseases. The activities of other en-

zymes of the energy-yielding metabolic pathways and the utilization of substrates in the muscles of alcoholics are not yet known accurately.

The purpose of the present investigation was to study some parameters of energy metabolism in skeletal muscle of chronic alcoholics. The activities of CPK, hexokinase (HK), LDH, malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) were measured 1- days after a drinking period after 6-7 days abstinence and after one month's physical conditioning. In addition the consumption of muscle glycogen during physical exercise was examined.

MATERIAL AND METHODS

Subject and experimental arrangements. Two experiments were performed. In experiment I the examinations were made on ten voluntary 4-53-year-old chronic male alcoholics. The muscle biopsies and the ergometer tests were performed after 6-7 days abstinence and after 1 month physical conditioning. The control group consisted of 16 habitually fit men, aged 13- years (Table I).

The physical conditioning of the alcoholics was started after the first examination and consisted of daily training of about 2 hours including jogging, oblique ball, swimming and gymnastics, varying according to the actual physical fitness of the subjects. All physical working capacity (PWC) measurements were performed under controlled condition between 9 and 11 a.m. after light breakfast. The subjects avoided vigorous physical activity before the test. The muscle biopsies were taken both at rest and immediately after the PWC_{max} test, which consisted of 3 different work loads on bicycle ergometer during 12 min. The work loads were estimated to be equal for every subject and control in relation to

Table 1 Physical fitness, enzyme activities, initial glycogen concentration and consumption in skeletal muscle of conditioned alcoholics and controls

I=before, II=after conditioning

	Alcoholics		Controls		p			
	I	II	I	II				
	n	Mean±S.E.M.	n	Mean±S.E.M.	Mean±S.E.M.	I-II	Alc I-contr	
PWC ₁₇₀ (kpm/min)	10	810± 68	10	995± 70	16	1164± 42	<.01	<.001
CPK (U/mg protein)	4	11.66±0.18	4	11.49±2.16	4	10.89±1.36	n.s.	n.s.
HK (mU/mg protein)	4	3.77±0.58	4	4.14±1.25	4	3.81±0.76	n.s.	n.s.
LDH (mU/mg protein)	4	618± 155	4	633± 762	4	570± 70	n.s.	n.s.
MDH (mU/mg protein)	4	938± 78	4	977± 45	4	1068± 48	n	n.s.
SDH (mU/mg protein)	4	20±0.33	4	1.77±0.30	4	1.88±0.16	n.s.	n.s.
Glycogen ^a	9	14.20±1.27	9	12.20±1.1	4	11.77±0.48	n.s.	n.s.
Glycogen decrease during exercise ^b	9	3.64±0.79	5	3.40±0.40	11	1.78±0.25	n.s.	<.05

g/kg muscle wet weight. mg/kg muscle wet weight related to work and body weight (kpm/kg)

weight and physical fitness. The enzyme activities and glycogen concentration were measured from every muscle specimen.

In experiment II six voluntary 26–47 year-old chronic male alcoholics were examined 1–2 days after an acute drinking bout. They were selected from the Alcoholic Consulting Center immediately after their admission. Six sedentary healthy men of corresponding ages served as controls. The muscle biopsies were taken only 1 rest and were used for the analysis of the enzyme activities. The experiments were arranged so that the analyses of the muscle specimens of one alcoholic and his control could be performed simultaneously.

Muscle biopsies. The muscle specimen (10–50 mg) taken from the distal part of m. vastus lateralis with needle (Stille-Werner). The specimen was immediately freed from blood and connective tissue and divided into two pieces, which were then weighed with an automatic balance (Mettler H20T). One piece was homogenized in 1.75 ml ice-cold tris-HCl buffer (pH 7.5) for enzyme analyses. The other piece was deproteinized in 0.4 ml 3 M HClO₄ for subsequent glycogen determination.

Enzymes. The muscle homogenate was centrifuged at 4°C for 10 min at 3000 rpm and the supernatant was used for the determinations. CPK was determined with Blochman Test Combination (Boehringer/Mannheim). HK according to Salberger et al. (13). LDH according to Kornberg (5). MDH according to Ochoa (10) and SDH according to Earl and Kornberg (1). The SDH activity possibly represent only a part of the total activity because of the insufficient homogenization. The enzyme activities were referred to supernatant protein which was analysed by a modified method of Lowry et al. (6). Somewhat different methods were used in the two experiments, e.g. in regard to the interval between the withdrawal of the muscle specimen and the enzyme analyses. Therefore the enzyme activity levels of the two experiments are not comparable. The methodological variation in duplicate analyses was less than ±5%.

Glycogen. For glycogen determination the sample was neutralized with 2 M K₂HCO₃ (to obtain a final pH of 7.8–8.5) and centrifuged for 10 min at 3000 rpm. The sediment was then hydrolyzed in 1 ml 1 M HCl at 100°C for 4 hours. From the hydrolyzate glycogen was determined as glycosyl unit by the o-toluidine method described by Hyvärinen and Nikkila (4).

All spectrophotometrical measurements were performed with Unicam SP 1800 Spectrophotometer.

Statistical methods. Standard procedures were used to calculate means and S.E.M. The significances of the differences between the means were calculated with Student's *t*-test or its modification for paired variables (3).

RESULTS

The enzyme levels of the chronic alcoholics who had been abstinent for 6–7 days (experiment I) did not differ from those of the controls. Neither did one month's physical conditioning change these enzyme values (Table 1).

The physical fitness of the alcoholics was significantly lower than that of the controls ($p<0.001$) measured with the PWC₁₇₀ test both before and after the physical conditioning. PWC was however significantly improved during the conditioning (Table 1).

The basal muscle glycogen levels of the alcoholics and the controls were about the same but the muscle glycogen concentration during physical exercise was decreased more ($p<0.05$) in the alcoholics. The conditioning did not diminish the difference in the efficiency of physical work between the two groups as judged from the utilization of muscle glycogen (Table 1).

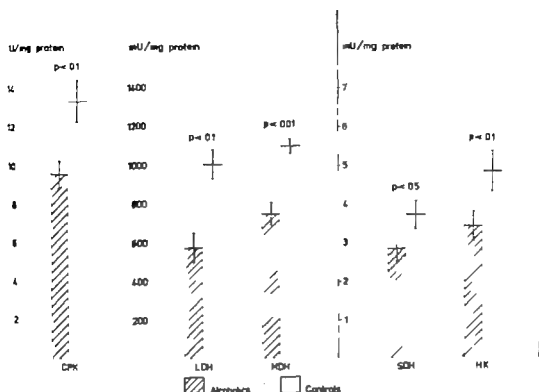


Fig. 1. Muscular enzyme activities (mean \pm S.E.M.) of the chronic alcoholics 1 day after a drinking bout and of the controls ($n=6$).

The results of experiment 1 prompted us to analyse the enzyme activities immediately after an acute drinking bout. In this experiment (Fig. 1) the activities of the measured enzymes were systematically lower in the alcoholics in every six pairs of subjects ($p < 0.05-0.001$).

DISCUSSION

The results of the present investigation showed that the activities of the measured enzymes of energy metabolism are decreased in the muscles of chronic alcoholics immediately after an alcoholic debauch (Fig. 1). It has been suggested that the permeability of the cell membrane is increased in damaged muscle cells following an alcoholic debauch and that the muscle cells consequently lose their intracellular enzymes into the extracellular space (7). The increased concentrations of muscular enzymes in serum (7-10) and the decreased enzyme activities in muscles support the suggested

increase of the permeability of cell membranes in alcoholics. The disturbances may however be unspecific and also occur in other acute diseases like the reduced LDH-5 content in skeletal muscle found by Nygren (8). Obviously the decreased enzyme activities observed in this study cannot be due to the possible physical inactivity of the alcoholics because there were no significant differences in enzyme values between the alcoholics after 6-7 days abstinence and the respective controls in spite of the significant difference of physical fitness.

Because the enzyme level in the muscles of the alcoholics, who had been abstinent for 6-7 days, was approximately the same as that of the control subjects (Table 1), it is obvious that one week is enough to normalize the enzymes of the muscle metabolism. Nygren (7) noticed that the serum CPK values returned to normal within 5-7 days after the alcoholic debauch had terminated. The fast recovery of the activities of metabolic enzymes of the muscle cells is also supported by the

fact that four weeks' conditioning did not cause any more changes in the enzyme levels.

It has been found that, during exercise, trained subjects are able to utilize fat more efficiently than untrained (7, 1). The difference in glycogen consumption between the alcoholics and the controls found in this investigation could be due to the better physical fitness of the controls (Table I) or possibly to shifting of the utilization of substrates in the skeletal muscles of the alcoholics towards carbohydrates. Because no major differences were observed in the initial glycogen concentrations (Table I) it can be supposed that dietary factors are not responsible for the difference in glycogen consumption.

Whether similar effects of a heavy drinking bout to those observed in the present study of chronic alcoholics can also be demonstrated in normal subjects will be the object for another investigation.

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DIAGNOSTIC ERRORS DISCOVERED AT AUTOPSY

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Abstract It has been questioned whether routine autopsies are needed any longer for control and correction of causes of death, specially in "lean-cut" cases. This question was therefore studied in connection with deaths in department of internal medicine. Among 400 consecutive deaths autopsy was performed in 383 (96%). Causes of death diagnosed before autopsy were compared with those established by the same clinicians after autopsy. A main cause of death the clinical diagnosis was thereby confirmed as correct in 57% of cases, and as erroneous in 30% of the remaining 13%. It had not been possible to make definite diagnosis ante mortem. Fewer diagnostic errors were encountered among patients below than above 70 years of age. There were also fewer errors when clinical diagnoses had been considered fairly certain than when estimated only as probable. However, even in the case of deceased patients below 70 years of age with fairly certain diagnoses, 15% were revealed to be erroneous at autopsy. The main cause of death was circulatory disorder in 67% of cases, and neoplastic in 17%. All other groups of diseases together accounted for the remaining 16% of deaths. Clinical diagnoses of neoplasms were more seldom found to be erroneous than diagnoses of other groups of diseases. Contributory causes of death were clinically underestimated. Of the disorders established as contributory after post-mortem 46% had been unrecognized before death. It is concluded that autopsies are still needed for control and correction of cause of death, also in "lean-cut" cases.

In connection with studies of the jurisprudence relating to hospital autopsies in Sweden a need appeared for evaluation of their practical importance (5-6). Earlier it has been stressed repeatedly that frequent post-mortems are necessary for control and correction of causes of death (7-21, 22). Lately though it has been questioned whether routine autopsies are of value in this context any longer, especially as regards clinically "lean-cut" cases (8-9). The aim of the present study was to elucidate this question on the basis of deaths occurring in a department of internal medicine.

DEFINITIONS

Underlying cause of death is defined by WHO as the disease or injury which initiated the train of morbid events leading directly to death. (26) This cause has in the past also been described as "primary cause of death", "principal cause of death" and is of ten equivalent to the expression "main diagnoses" used in clinical contexts (1). Generally the underlying cause of death is the condition entered on the lowest used line of Part I of the death certificate.

Contributory cause of death Complications or other than the underlying disease mentioned on the death certificate.

Clinical diagnosis Clinical diagnosis of the underlying cause of death when nothing else is stated.

Final diagnosis Diagnosis of the underlying cause of death as evaluated after autopsy when nothing else is stated.

ICD International classification of diseases 9th Revision (26). This classification contains 17 chapters, in the following referred to as main groups of diseases (e.g. VII. Diseases of the circulatory system). The main groups are further divided into sections, here named subgroups (e.g. Cerebrovascular disease), containing varying number of separate categories, diagnoses, each assigned a specific code number (e.g. Subarachnoid haemorrhage ICD 430).

MATERIAL AND METHODS

Serafimerlasarettet is university hospital which at the time of the study served an undefined population in Greater Stockholm. The study covers 400 consecutive deaths (8%) among 4895 in-patient admitted to the Medical Department during the period Jan. 16, 1970-June 16, 1971.

Every weekday morning during the survey period the author visited the wards in which patient had died during the previous day(s). Data about the deceased including history and medical findings, were collected in collaboration with the physicians responsible for each particular patient. All records were gone through. The cause of death as judged from the clinical point of view as registered on the death certificate.

The cases were divided into three groups according

fact that four weeks conditioning did not cause any more changes in the enzyme levels.

It has been found that, during exercise, trained subjects are able to utilize fat more efficiently than untrained (1). The difference in glycogen consumption between the alcoholics and the controls found in this investigation could be due to the better physical fitness of the controls (Table I) or possibly to shifting of the utilization of substrates in the skeletal muscles of the alcoholics towards carbohydrates. Because no major differences were observed in the initial glycogen concentrations (Table I) it can be supposed that dietary factors are not responsible for the difference in glycogen consumption.

Whether similar effects of a heavy drinking bout to those observed in the present study of chronic alcoholics can also be demonstrated in normal subjects will be the object for another investigation.

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Table V *Contributory causes of death as evaluated before and after autopsy*

Diseases, main groups ICD	Contributory causes of death as evaluated before autopsy			Contributory causes of death as evaluated after autopsy		
	Total no.	No	% of total	Total no.	No	% of total
Infectiv I	7	—	—	11	4	36
Neoplastic II	8	1	—	22	15	68
Endocrine, metabolic blood, mental, nervous system, III-VI	79	1	1	87	9	10
Circulatory VII						
Acute myocardial infarction	3	1	—	77	25	33
Chronic ischaemic heart disease	134	1	16	135	22	16
Other heart diseases	28	4	18	54	31	57
Cerebrovascular disease	31	5	16	53	77	51
Pulmonary embolism	5	3	—	52	40	96
Other vascular diseases	45	1	2	104	60	58
Respiratory VIII						
Pneumonia	58	21	36	79	42	53
Bronchitis	30	4	13	29	3	10
Digestive IX	15	—	—	55	40	73
Remaining groups, X-XVII	46	9	20	61	4	39
Total	489	7*	15	769	35*	46

plicating factors may also be of interest. If they are not discovered or are misjudged, the consequence may be inadequate treatment which might at least worsen the immediate prognosis for the individual.

There are several earlier surveys concerning the total frequency of diagnostic errors as estimated from autopsy materials. These are summarized in Table VI. Due to several circumstances recounted below, however, the results are not easily comparable with those in the present study.

Period studied. Compared to early studies the disease panorama, diagnostic facilities and modes of treatment have changed. Conditions might also differ in different countries.

Errors regarding major or underlying disorder. Nine of the 14 authors referred to in Table VI have not declared whether they considered only inaccuracies regarding the underlying disease or whether they also included more or less important secondary findings in their figures. Where the principal disease was the subject for study, rules for its selection have often been omitted.

Apart from the present study only two others (11, 14) have mentioned that the *clinical diagnosis* was ever *uncertain or unknown*. In Sweden autopsy is compulsory by law in such cases which occurred with similar frequency in the two previous and the present surveys (1–15%). In one of them (14) as in the present one, the unknown cases

were handled separately. In the remainder all clinical diagnoses, including pure guesses, were included when calculating the total rate of inaccuracies, which should give comparatively higher figures.

Autopsy rate. When autopsy is not performed in almost every case, the material becomes selected. Deceased patients of younger ages, certain disorders and generally "difficult cases" are overrepresented (17) which most influence the frequency of inaccurate diagnoses established at post mortem. There is only one other survey (20) with an autopsy rate on the same high level and accordingly with a similar minimum of such selection.

Clinical diagnosis established retrospectively. In all except one (11) of the materials referred to in Table VI the clinical diagnoses were worked out retrospectively from patient records or autopsy request forms and might therefore be dubious. Pathologists and other investigators not familiar with the clinical picture in a case may also tend to overestimate autopsy findings. To avoid these objections in this study the evaluations were made by the same clinicians before and after autopsy, which was not done in any of the other studies.

Subject for study and size of material. The aim of most of the major studies was to evaluate the validity of mortality statistics. Inaccuracies due to mistakes made in death certificates or in the central coding were often inseparable from those due to

Table VI Frequency of diagnostic errors as estimated from autopsy materials a comparison between previous studies and the present one

Ref no	First mentioned author	Study period or year of publication	Errors regarding main or underlying disorder	Clin. diagnosis unknown or uncertain (% of cases)	Autopsy rate (%)	Clin. diagnosis established retro- or prospectively	Size of material	Total rate of diagnostic errors (%)
15	Karsner	Publ 1919	—	—	20	Retro-	600	68
4	Swarnott	1933-37	—	—	40	Retro-	8 080	21
25	Wallgren	1934-39	—	—	7 (low)	Retro-	1 000	49
18	Munck	1940-49	+	—	78	Retro-	1 000	20
10	Gruver	1947-53	—	—	76	Retro-	1 106	6
13	James	1951-52	+	—	24	Retro-	1 889	48
3	Bornn	Publ 1952	—	—	50	Retro-	1 078	33
16	Landes	1954-64	—	—	48	Retro-	1 132	48
27	Wilhom	1958	—	—	53	Retro-	265	47
14	Justam-Besançon	1958-61	—	15 (excluded)	79	Retro-	1 000	30
11	Hessman	1959	+	12 (not excl.)	63	Pro-	9 501	55
20	Otterbom	1960	+	—	98	Retro-	377	28
III	Schulz	1967	+	—	18	Retro-	4 652	52
1	Holler	Publ. 1970	—	—	50	Retro-	200	48
	Present study	1970-71	+	13 (excluded)	96	Pro-	303	30

diagnostic errors. Furthermore deaths at all ages and of all causes were included. However at least in the more recent surveys circulatory disorders predominated followed by neoplasms as in the present material.

Bearing all these differences of approach in mind it is not surprising to find the total rate of errors listed in the studies reviewed to vary from 10 to 68% the present findings of 30% being among the lowest. Beyond all doubt anyway it can be concluded that autopsies earlier did and still do reveal a considerable number of errors in clinical diagnoses of patients dying in hospitals.

There is no convincing sign that the rate of errors has diminished over the years although the diseases and types of mistakes have changed. Part of the explanation might be that the results of gradually improved diagnostic facilities are cancelled out by a gradually increased proportion of patients of high age among whom diagnostic errors are more frequent.

It seems natural and is in accordance with other authors' findings (11) that greater difficulties and more inaccuracies exist in diagnoses of elderly patients. Their symptoms are often blurred and the presence of multiple diseases further complicates the picture (28).

The clinician's judgement of the certainty of

diagnosis turned out to be a good measure of its risk of inaccuracy which was also found by Hessman and Lipworth (11). Thus when the diagnosis was estimated as fairly certain it was revealed to be inadequate in 5% of cases but in 45% when considered only probable. In the latter category the diagnosis must have been open to question, which seems reassuring. The figure of 25% errors in what may be labelled clinically clear-cut cases appears more remarkable. Obviously such cases cannot be excluded from autopsy on the grounds that errors in clinically stated causes of death are seldom or ever revealed. When no autopsy can be performed though it might be of value for future investigations if certainty of clinical diagnosis was stated on the death certificate. This information together with the age of the patient could be used for a rough estimation of the risk of error of the main diagnosis.

Whether clinical diagnoses of the cause of death are certain or uncertain, is there any point in continuing to discover which are erroneous by means of routine autopsies? In the author's view there are many arguments in favour of doing so and some of them will be listed here.

For clinicians it has a great postgraduate educational value to have diagnoses checked in each case of death. Disclosing of mistakes stimulates

critical reasoning and increases the willingness to reconsideration, which should in turn improve future patient care. With experience from an increasing number of cases more systematic observations can be made which are applicable in current clinical practice. Since the disease panorama changes, as do modes of treatment and diagnostics, the acquisition of such knowledge must be continuous. The experience of clinical diagnostics gained from the present material will be described in a subsequent report (4).

The post-mortem check of clinical diagnoses is important also for reasons other than the post graduate education of doctors that it offers. If autopsies were not performed the central registration of causes of death for individual subjects would be affected. This would also be the consequence as regards the Cancer Registry and local records and statistics.

Thus, without autopsies 7% of the present cases would have been classified as belonging to a wrong main group of diseases in such registers as regards their underlying cause of death. Another 7% would have been assigned to an erroneous subgroup of disorders, and 16% to inaccurate ICD numbers within the right subgroups. Almost half of the disorders that contributed to death would not have been recorded at all.

However, in mortality statistics, the total number of deaths ascribed in a certain cause would not be affected if clinical overdiagnoses and underdiagnoses outweigh one another. In the present material this was the case to some extent as regards the underlying cause of death when main groups of diseases were considered. The neoplastic group, though, was clinically somewhat underestimated whereas there were a few net overdiagnoses of most other groups of diseases.

Furthermore, in the major study of mortality statistics from England and Wales earlier referred to (11) it was concluded that even the total figures would be improved if frequent post-mortems were performed. Certainly contributory causes of death would be markedly underestimated without autopsies as judged from the present material.

Also to enable studies and follow-ups of disease processes in individual patients it is essential that causes of death are correct in each case. This concerns both the central registration and that in records and statistics of each hospital. It is of little help in scientific studies to know that pos-

sible errors among investigated non-autopsied subjects are probably outweighed by other errors in other subjects.

For relatives of the deceased the importance of knowing the correct cause of death, at present or in the future, must not be underestimated. Furthermore, when on rare occasions contagious diseases are disclosed, it is of direct practical importance that the relatives are themselves examined.

Thus it is concluded that diagnostic errors are still discovered at routine autopsies and that the continuous control and correction of causes of death hereby obtained is important.

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CLINICAL DIAGNOSTICS EXPERIENCE FROM 383 AUTOPSIED CASES

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Abstract The aim of the present study was to investigate whether the experience of clinical diagnostics could still further be enriched through routine autopsies. The question was studied by comparing diagnoses made by the same clinicians before and after autopsy in 383 subjects. Clinical misinterpretation thereby revealed were further analysed and described. Acute myocardial infarction (AMI) was the most common cause of death. The diagnosis was seldom disproved when clinically considered fairly certain, but the disorder had often been missed and mistaken, especially among patients with known chronic ischaemic heart disease (IHD). Hidden behind this latter label were also cases with valvular lesion or with lung disorders and right heart failure. Apart from chronic IHD cerebrovascular diseases were initially overdiagnosed as main cause of death, sometimes recent myocardial infarcts or existing neoplasm were instead disclosed at autopsy. Clinical diagnoses of neoplastic disorders were seldom found to be erroneous, but misdiagnosis should be more often considered clinically. In several cases where it had been impossible to establish definite diagnosis on clinical grounds acute abdominal disorders were revealed post mortem. An increased suspicion as regards these diseases seems warranted in obscure cases. The misinterpretations were frequently a consequence of our tendency to stick to earlier diagnoses and to overlook the development of new signs and symptoms. The same mechanism might partly explain why disorders contributing to death had often been unrecognized clinically—most frequently pulmonary embolism, AMI, carboids of liver and ulcer of stomach or duodenum. It is concluded that the Latin epigram regarding autopsies is still valid: *Mortui vivis docent*—let the dead teach the living.

In recent years the value of routine autopsies has been questioned (7-9). In a previous paper (5) it was concluded that they were still important for control and correction of causes of death also in what appeared to be clear-cut cases. The aim of the present study was to investigate whether in addition, the experience of clinical diagnostics

could still further be enriched through such or dummy post-mortem examinations. An analysis and description are therefore given of cases where clinical misinterpretations were disclosed at autopsy.

MATERIAL AND METHODS

In the Medical Department of Serafimerlasarettet 400 deaths occurred during the period Jan 16 1970–June 16 1971. The present survey covers the 383 cases (96%) in which autopsy was performed. Of the deceased patients 203 were men and 180 women. Their mean age was 70 years. Detailed descriptions of material and methods as well as definitions are given in previous paper (5).

RESULTS

The results will be presented under different headings according to the main group of diseases to which the clinical diagnosis belonged in the 333 cases in which a diagnosis had been made. *Circulatory disorders* predominated as underlying causes of death and will therefore be described first. Next in size and order of presentation is the group of *neoplastic disorders*. Thereafter the other groups of diseases are described together under the heading *miscellaneous disorders*. The 90 cases in which there had been insufficient clinical evidence for a definite cause of death to be established are presented as *cases with clinically unknown causes of death*. Finally diagnoses of *contributory causes of death* will be dealt with.

Circulatory disorders as underlying cause of death
Clinical diagnoses of circulatory disorders were established in 228 cases. The final diagnoses for 218 of them were still within the same main group

Table I. *Circulatory disorders. Correlation between clinical and final diagnoses as regards the underlying cause of death*

Clinical diagnoses		Final diagnoses							Total no.	
		Circulatory disorders								
Disease groups, code nos.	ICD	Total no	394-396	410	412	434-421 428	430-438	440-448	450-453	
Circulatory disorders		228								218
Rheum. heart disease 394-396		11	10			1				
AMI 410		85	1	74	2			2	3	
Chronic IHD 412		56	2	25	20	2	1	1		
Other heart disease 404 421-428		10		3	1	6				
Cerebrovascular 430-438		61		3	1		55			
Arterial 440-48		3	1					2		
Venous thrombosis pulm. embolism, 450 453		2							2	
Disorders other than circulatory		105	2	1		3	2		1	9
Total		333	16	104	24	14	58	5	6	227

Figures in *italics* indicate correct clinical diagnoses.

of diseases. In the remaining 10 cases disorders other than circulatory were revealed at autopsy (Table I). Within the circulatory subgroups chronic ischaemic heart disease (IHD) and cerebrovascular diseases were more often over than underlying nosed clinically. The opposite was true for acute myocardial infarction (AMI) rheumatic other heart disease arterial and venous affections.

As regards the *rheumatic heart diseases* all clinically diagnosed were confirmed at necropsy. In one patient, however, a missed active non-rheumatic endocarditis was considered the main cause of death. In four cases rheumatic valvular lesions had clinically been misinterpreted as general arteriosclerosis or IHD (Table I).

In most of the 85 cases in which AMI was clinically considered to be the underlying cause of death there was no revision after autopsy except for 11 cases. In four of the latter the clinical diagnosis had been considered probable and in seven fairly certain.

A probable diagnosis of AMI had been made for two middle-aged men who had been admitted as emergency cases with severe central chest pain. One of them had ST elevations consistent with posterior wall infarction. Both patients died in shock a few hours after admission. The one with ECG changes had venous thrombosis and massive pulmonary embolism, the other had an aortic rup-

ture. The other probable diagnoses were made on two elderly women with multiple diseases who developed ECG changes suggestive of AMI some days before death. Further evidence of the diagnosis was lacking. In one advanced coronary constrictions were proved but no myocardial necrosis. In the other there was a small infarct the main disease however turned out to be an infective haemorrhagic enterocolitis (Table I).

In four of the seven patients with a clinical diagnosis of AMI considered fairly certain, recent infarct (<8 weeks) was also verified by the pathologist. On the basis of other post-mortem findings, however, this disease was estimated only to have contributed to death. The underlying disorders in these cases were reassessed as acute haemorrhagic pancreatitis severe aortic stenosis aortic rupture and disseminated lung cancer. The remaining three cases with fairly certain diagnosis were women 87, 86 and 77 years old. They were initially treated in the Coronary Care Unit and the diagnosis of myocardial infarction was made according to conventional criteria, based on history, enzyme curves and ECG (18). They all died about 3 weeks after admission. At autopsy none was found to have a recent infarct. One had an old scar and her main disease was considered coronary although she also had a small rectal cancer. The other two had massive pulmonary embolism and

Final diagnoses

Disorders other than circulatory

Infective	Neoplastic	Respiratory	Digestive	Total no.
2	6	1	1	10
1	1		1	3
1	3	1		5
	2			2
				96
				106

multiple haemorrhagic infarcts in the lungs. The sources of the emboli could not be definitely established.

Chronic IHD was clinically estimated to be the main cause of death of 56 patients. In 20 of them this diagnosis was upheld after the post-mortem investigation and in 36 other causes of death were revealed (Table I).

Of the latter as many as 25 were found to have recent myocardial infarction which had not been diagnosed for several reasons. 1) The majority of patients were in a late stage of chronic IHD treated in the hospital for heart failure. In some cases the myocardial infarction had occurred on one of the last days before death and had not considerably contributed to the patients' already severe symptoms. The diagnosis was therefore not suspected. On other occasions the results of irregular ECGs and enzyme determinations had caused the diagnosis to be ruled out as not very probable. 2) Some patients with known chronic IHD died so soon after arrival in the hospital that time did not permit collection of evidence for the diagnosis of AMI. 3) On some occasions patients with chronic IHD were admitted for treatment of increasing heart failure. Due to absence of chest pain complaints the correct diagnosis was not suspected.

Chronic IHD was also overdiagnosed at the expense of circulatory disorders other than myocardial infarction. Two patients, above mentioned, had rheumatic heart disease. Two had myocardial degeneration of non-coronary aetiology. One severely deteriorated woman had multiple cerebral softenings and a subarachnoid haemorrhage. One man had recent thrombotic occlusions of the renal arteries.

In 5 cases with clinical diagnosis of chronic IHD the post-mortem revealed disorders in main groups of diseases other than circulatory. Sequelae of pulmonary tuberculosis had caused restriction of lung function and right heart failure in one patient. Chronic bronchitis with severe emphysema had caused similar symptoms in another. Two elderly patients treated for heart failure turned out to have pulmonary and pleural metastases, the primary site of malignant growth was stomach and rectum, respectively. The fifth patient arrived at the emergency ward with central chest pain and IHD was considered a probable diagnosis. He died within 3 hours and autopsy disclosed a widespread undifferentiated lung cancer.

In 61 patients the clinical diagnosis of a *cerebrovascular disease* was made, verified at autopsy in 55 (Table I). The exact type of vascular disease was not specified clinically in 50% of cases. The six erroneously diagnosed were among them. Cases 1-3 had large recent myocardial infarcts which were considered to be the main cause of death. One of these patients also had small old softenings in the brain. Another had cerebral infarct, the age of which accorded with the time of her initial moderate symptoms. The myocardial process had appeared later, causing deterioration and finally death. In the third patient the brain was not at all affected nor were the carotid arteries.

Case 4 had multiple cerebral emboli apparently dislodged from the heart. Chronic IHD was judged to be the main cause of her death. Cases 5 and 6 in one woman malignant glioma was discovered, and in one woman widespread metastases from breast cancer operated upon many years earlier.

There were 15 cases with clinical diagnoses of main cause of death in the remaining circulatory subgroups. In *other heart arterial and venous diseases*. For 14 of them there was no revision of the diagnosis after autopsy (Table I). For the following three there was. Alcoholic heart disease was believed to be the cause of longstanding cardiac arrhythmias in one middle-aged man, and an obscure myocardial disorder to be responsible for

Table II *Neoplastic disorders* Correlation between clinical and final diagnoses as regards the underlying cause of death

Clinical diagnoses			Final diagnoses							Disorders other than neoplastic
			Neoplastic disorders							
Disease groups, code nos.	ICD	Total no.	150-157	162	174	183	200-209	183 186-193	Total no.	
Neoplastic disorders		50							48	
Digestive organs 150-157		8	8							
Bronchus or lung, 16..		10		9					1	
B. east, 174		4			4					
Prostate 185		6				5			1	
Lymphatic or blood system, 200-209		12					12			
Various 183 186-193		6						6		
Unspecified site 196-199		4		3	3					
Disorders other than neoplastic		283	4	4	1		1	1	11	
Total		333	13	16	5	5	13	7	59	

Figures in italics indicate correct clinical diagnoses.

similar symptoms in another. Both turned out to be victims of chronic IHD: one had had a recent infarct as well. In one woman rheumatic heart disease had been clinically misinterpreted as general arteriosclerosis.

In nine subjects earlier believed to have died of other illness, circulatory diseases were disclosed post mortem (Table I). One had had a clinical diagnosis of neoplastic disorder and eight had had different diagnoses among miscellaneous disorders.

Neoplastic disorders as underlying cause of death

Clinical diagnoses of malignant disorders were made in 50 cases. In 48 of them the final diagnoses still belonged to this group of diseases (Table II). The diagnoses of these subjects also remained within the same subgroup, except in 4 cases in whom the primary site of malignancy had been unknown before death. In three of the latter it turned out to be in the bronchus, and in the fourth in the large intestine.

Table III *Miscellaneous disorders* Correlation between clinical and final diagnoses as regards the underlying cause of death

Clinical diagnoses				Final diagnoses								
				Miscellaneous disorders								
Disease groups	code nos.	ICD	Total no.	1 136	40- 279	280- 389	460- 519	520- 577	580- 629	710- 796	800- Total no.	
Miscellaneous disorder			55								42	
Infective 1-136			5	1					1			
Endocrine, metabolic 40-279			4		4							
Blood, mental, CNS 280-389			8	1		5						
Respiratory 460-519			8				5					
Digestive 520-577			14					12				
Genitourinary 580-629			7						6			
Connective tissue, symptoms 710-796			3						1	1		
Violent causes, 800-			6								5	
Circulatory disorders			228	2			1	1			4	
Neoplastic disorders			50					2			1	
Total			333	4	4	5	6	14	8	1	47	

Figures in italics indicate correct clinical diagnoses.

In two of the eight subjects with malignant neoplasms of digestive organs the tumour had clinically not been correctly located within the gastrointestinal tract. Instead of the pancreas the gall-bladder was the primary site in one case. In the other case the growth had started in the oesophagus and not in the stomach as was believed.

The two deceased patients in whom the clinical diagnosis of malignant neoplasm was disproved at autopsy had the following histories.

Case 7 A 89-year-old man had urinary trouble. His prostatic gland was hard and asymmetric. Irregularities suspect of being metastases were seen on X-ray of the skeleton, and disseminated prostate cancer was considered his main disease. He died in bleedings from stomach ulcer. At autopsy there were no sign of cancer nor in the subsequently examined microscopic preparations. The ulcer was therefore the underlying cause of death.

Case 8 A 78-year-old woman suffered from fever, malaise and dyspnoea. She had pleural effusion, enlarged hilar lymph nodes and tumour-like structure in the right upper pulmonary lobe. Autopsy revealed severe mitral stenosis, widespread embolism and heart failure as her main disease. Instead of the suspected bronchial cancer inactive tuberculous lymph nodes had caused stenosis of the right upper bronchus and there was an abscess peripherally to it.

Of the eleven missed cases with malignant neoplasms (Table II) six had had clinical diagnoses of *circulatory disorders*. They were dealt with under the previous heading. The remaining five cases will be presented according to their clinical diagnosis among *miscellaneous disorders*.

Miscellaneous disorders as underlying cause of death

Clinical diagnoses in main groups of diseases other than the circulatory or neoplastic had been made in 55 patients (Table III).

Septicaemia was the only infective disease diagnosed clinically as underlying cause of death. This was upheld after autopsy in only one of five cases. In three the site of infection and accordingly the underlying disorder was discovered. This was pyelonephritis with multiple abscesses in one case and acute endocarditis in two. In a fourth case no signs at all of septicaemia were found and blood cultures had been negative. Instead venous thromboses and pulmonary emboli revealed at autopsy were considered to have caused death and the septic temperature elevations noted before death.

In the other groups of diseases listed in Table III the clinical diagnosis was mostly confirmed at autopsy. Six of the cases (nos. 9-14) in which it was not will be further described.

Case 9 A 99-year-old woman had been thoroughly investigated during several years in different hospitals, and the diagnosis of sideroblastic anaemia was established. She was treated in this hospital during the last months of her life, during which time she gradually deteriorated with bouts of fever. Several antibiotics and 4-week course of antituberculous treatment were tried without any effect. Her main disease was therefore considered to be the above complicated by intercurrent infections. Post-mortem examination however revealed solitary tuberculous lesion in the spleen, liver and bone marrow.

Case 10 A middle-aged man was treated for Coombs-positive severe haemolytic anaemia of unknown aetiology. He died in haemolytic crisis six days after admission. A reticular cell sarcoma in mediastinal lymph nodes, spleen and liver was discovered at autopsy.

Case 11-12 Two heavy smokers with chronic bronchitis admitted long function, turned out to have disseminated bronchial carcinoma.

Case 13-14 Two patients with cirrhosis of the liver primary liver cancer had developed, explaining the deterioration for which they were admitted to the hospital.

In four cases clinical diagnoses of *circulatory disease* were revised after autopsy to miscellaneous disorders. Two of them had final diagnoses of infective disorders (infective colitis, tuberculosis), one of respiratory (chronic bronchitis) and one of digestive (pancreatitis). These cases were described above. The history of a man in whom prostate cancer was disproved and gastric ulcer

Final diagnoses

Circulatory disorders	Neoplastic disorders
8	5
3	
1	1
1	2
1	2
1	
1	
18	6
1	48
227	69

was instead revealed as underlying cause of death (case 7).

Clinically unknown causes of death

In 50 patients (13% of all) there had been insufficient clinical evidence for any definite cause of death to be assigned. Twenty-six (52%) of these patients had been hospitalized for less than 72 hours before death. The diagnoses made after autopsy are given in Table IV. Circulatory diseases predominated as among deceased patients with established clinical diagnoses. Instead of neoplasms, however, digestive disorders were the second most common cause of death among these clinically unknown cases.

Recent myocardial infarcts were disclosed in 15 cases. None of them had had any previous history of IHD. They died either soon after arrival in the hospital or suddenly and unexpectedly while hospitalized for other non-lethal conditions. Therefore it was impossible to make a diagnosis on clinical grounds. Similar circumstances existed in three cases in which dissecting aortic aneurysm was disclosed at autopsy.

Of the ten cases in whom digestive diseases were revealed some will be mentioned. Two of them, previously healthy, were admitted with ascites and a disease history of less than one week. One died a few hours after arrival and the other

Table IV. Diagnoses of the underlying cause of death established after autopsy in 50 cases considered unknown before death

Diseases, code nos. ICD	Total	Hospitalized <72 h
Circulatory disorders	31	17
AMI, 410	15	8
Aortic aneurysm, 441	3	3
Chronic IHD, 41	5	2
Venous thrombosis with pulm. embolism, 453	2	—
Other	6	4
Digestive disorders	10	5
Cirrhosis of liver, 571	2	1
Cholelithiasis with cholecystitis, 574	3	1
Acute pancreatitis, 577	2	1
Intestinal adhesions with ileus, 560	1	—
Other	2	—
Neoplastic disorders	5	1
Other diseases	4	3
Total	50	26

within a week of hospitalization. Both turned out to have had cirrhosis of the liver; the former portal vein thrombosis as well. The six cases in which acute abdominal disorders, cholecystitis, pancreatitis or ileus were disclosed at autopsy had very similar histories. They were brought to hospital because of general deterioration, loss of appetite, fatigue, malaise and fever. None had any remarkable signs on physical examination, nor did they complain of abdominal pain. The conditions remained clinically obscure and the correct diagnoses were never suspected.

Contributory causes of death

Contributory causes of death were clinically underestimated. Thus of 769 disorders established by clinicians as contributory after autopsy, 46% had been overlooked before death. The most frequently missed diagnoses were pulmonary embolism, AMI, cirrhosis of liver and ulcer of stomach or duodenum of which 96%, 93%, 74% and 73% respectively were clinically missed.

The underlying diseases of patients in whom these contributory causes of death had been overlooked were of all types. However, all except one of the pulmonary emboli afflicted patients with neoplasms or circulatory diseases.

DISCUSSION

In the present study the causes of death were assigned on clinical grounds before autopsy and re-evaluated by the same clinicians and the author when post-mortem findings were revealed. The diagnoses made before and after autopsy were subsequently compared and the cases were analysed from a clinical point of view. The author has not been able to find any studies performed in this way which deal with all causes of death.

On several occasions autopsy results have been used for retrospective studies of one or more clinical diagnoses. Some of these will be referred to in the following, although the results are not strictly comparable owing to the different methods of study applied.

The same objection can be raised against comparisons with results from studies on the accuracy of mortality statistics. In such investigations, furthermore, the cases are not clinically analysed. Some observations may nevertheless be relevant

also from this point of view and are therefore referred to. This specially applies to findings in the major British investigation by Hensman and Lipworth carried out in 1959 (11), the only other study of this kind found in which clinical diagnoses had been made before autopsy.

In the Medical Department of Seraflimerlasarettet the most common cause of death was AMI. The diagnosis was seldom disproved when clinically considered fairly certain. The only misinterpreted disorder disclosed at autopsy under these circumstances was pulmonary embolism. Such misinterpretations have been described earlier (12-17) but seem to be possible even today on rare occasions in spite of coronary care units and defined diagnostic criteria. Also in more obscure cases aortic rupture or aneurysm must be considered. Further more the risk of failure in recognizing other and sometimes more important diseases in patients with correctly diagnosed myocardial infarctions must not be overlooked.

On the other hand AMI had quite often been missed clinically as reported by other authors (4). This had happened especially often among patients with chronic IHD. In epidemiological studies these two closely related entities are often combined into one group which of course neutralizes misinterpretations between the two diagnoses. In hospitals however the accuracy of each diagnosis is of practical importance since patients are treated differently according to which diagnosis is assumed.

Thus a high degree of suspicion of AMI seems to be warranted in patients with chronic IHD. Even in the absence of chest pain complaints infarctions should be looked for regularly and particularly when heart failure develops. It cannot be excluded that infarcts in such already severely diseased hearts might present somewhat atypical clinical signs. For practical purposes fixed diagnostic criteria should therefore be applied with care in order not to erroneously rule out the diagnosis. For scientific purposes though fixed definitions are often necessary. Liberal admission criteria in coronary care units might also be considered when deterioration occurs in patients with chronic IHD. However many of these patients are in such a late stage of disease that any measures would be fruitless.

Chronic IHD was, on the whole, often erroneously estimated to be the most important disorder of the patient. Careful reconsideration is therefore

necessary before the diagnosis is accepted as the main one. Apart from AMI already mentioned valvular lesions were hidden behind the label of chronic IHD as has been noted before (3-19). Furthermore breathlessness, pulmonary rules and right heart failure are not rarely explained by mechanisms other than backward failure due to chronic IHD. Thus disorders in the lungs like chronic bronchitis, sequelae of tuberculosis and cancers must be more often suspected in patients with these symptoms.

Cerebrovascular disorders were clinically overdiagnosed as reported by some authors (8-11) but not by others (14). Single cases of brain tumour and metastatic growth were instead revealed at autopsy. Large AMIs seemed to have caused pre-dominating cerebral symptoms in some elderly patients although no arrhythmias were noted. Probably this was due to a reduced blood flow caused by impaired cardiac output, in already arteriosclerotic brain vessels. Awareness of this symptomatology which has also been noted in retrospective studies (4), and routine ECGs might disclose the correct aetiology in similar cases.

Clinical diagnoses of neoplastic disorders were seldom found to be erroneous. On the other hand it appears from this material that malignant disorders should be more often suspected clinically. This is in accordance with the findings of Hensman and Lipworth (11). They concluded that especially cancers of the lung, and of gallbladder or bile ducts are frequently unrecognized clinically or misinterpreted as malignancies of other sites. Examples of such mistakes occurred also among the present few cases.

Among "miscellaneous" clinical diagnoses there were examples of missed disorders that are well known as offering particular diagnostic difficulties. Such were subacute endocarditis (6), miliary tuberculosis (16) and malignant lymphoma restricted to abdomen and mediastinum (15). The difficulty involved in diagnosing these conditions must be re-emphasized.

In many cases in which an underlying cause of death could not be settled on clinical grounds, this was simply because death had occurred suddenly and unexpectedly. It appears more surprising and also sad that in six cases acute abdominal disorders (choledocholithiasis with cholecystitis, ileus, pancreatitis) had not been revealed, although none of these patients died suddenly. Probably the level of

suspicion as regards acute surgical disorders is low in medical wards. Accordingly examinations relevant to the respective diagnoses are not carried out. Two of the six patients were examined also by experienced surgeons who nevertheless did not disclose the correct state of matters. Therefore in patients with vague symptoms like loss of appetite, malaise, fever and general deterioration acute abdominal disorders must be seriously reflected on and searched for since on some occasions they might be cured by means of surgical or other intervention.

The great majority of diagnostic errors fell upon patients with one or more clarified diseases. The tendency of clinicians to stick to earlier diagnoses and overlook the development of new signs and symptoms has been pointed out before (10). Evidently we need to be further reminded that one disease does not give immunity against all others. On the contrary it might predispose to some like cirrhosis of the liver to primary liver cancer and smoking bronchitis to lung cancer.

The tendency to overlook some of several simultaneous diseases in the same patient was apparent also as regards contributory causes of death. However the frequently non-diagnosed myocardial infarctions and pulmonary embolism were often terminal events in severely diseased patients. Death as expected and further investigatory efforts had ceased. On other occasions the named complications occurred in patients already suffering from circulatory disorders which caused obvious difficulties in differential diagnostics. Furthermore when the development of a lung process was recognized it was too often interpreted as pneumonia (5). Several authors have pointed out that pulmonary embolism is the most frequently missed complication among hospitalized patients (1). Nevertheless a greater attention to this disorder is still needed.

Overlooked peptic ulcers might also have been terminal caused by the stress of severe disease. More surprisingly even advanced cirrhosis of the liver had escaped clinical recognition both in cases where it was the underlying disorder and in those where it was contributory. It has been reported earlier though that symptoms and signs are often scanty (13). This of course increases the risk of their being blamed on other coexisting disorders not noticed at all or not until an agonal stage.

It is difficult to evaluate the consequences of

diagnostic mistakes for the individuals. However the general condition of the patient at the time of inaccurate diagnosis appears to be of the greatest importance. Misinterpretations between closely related diseases might be of great significance for a patient in good state. On the other hand major diagnostic errors may be unimportant in the case of severely ill patients since further investigations or treatments would be fruitless whatever the diagnosis.

In the present study on some occasions it might have been of real benefit for the patient if the correct disease had been revealed (i.e. primary tuberculosis, acute abdomen, malignant lymphoma). Great efforts had often been made to arrive at the truth but without success. It is hoped that opportunities for involved physicians to solve similar difficult problems in the future will be improved through the experience gained from these cases.

Otherwise the diagnostic errors in this material were made in most cases at an advanced stage of disease. Therefore they were of little prognostic importance for the individual patients. If it had been possible to disclose the correct diseases (i.e. neoplasms, endocarditis, valvular lesions) at an earlier point the outcome for some subjects might have been affected. Experience of diagnoses obtained through autopsies therefore should mainly be useful in the care of patients in earlier stages of disease.

Thus it appears evident that routine autopsies still provide a knowledge of clinical diagnostics which is useful in current practice.

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COLONY FORMING AND COLONY STIMULATING CELLS IN RELATION TO PROGNOSIS IN LEUKEMIA

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Abstract In order to assess the prognostic significance of colony-forming cells (CFC) and colony-stimulating cells (CSC) peripheral white blood cells from patients with leukemia were cultured in agar. Before treatment CFC were found in eight of 12 patients with acute myeloblastic or acute undifferentiated leukemia. The four patients who went into complete remission belonged to this group with CFC activity. Four patients without CFC initially died without remission. The CFC persisted during treatment, except in the event of severe drug-induced leukopenia. The colony morphology was of the large normal or the small abnormal type initially in remission the colonies were only of the large normal type. Cells with colony-stimulating ability were found in six of the 12 patients before treatment, there was no change in rate during treatment. These six patients included the four with complete remission. All the six patients without CSC died without remission. Since all the four patients with complete remission but only one of those without had both CFC and CSC initially it seems that this combination may be a favorable prognostic sign in acute leukemia, while the absence of CFC and/or CSC may indicate poor prognosis.

Colony-forming cells (CFC) can be demonstrated both in human bone marrow and peripheral blood using an *in vitro* culture system with a semisolid supporting medium (2, 12, 13, 17-21). The exact nature of the CFC is still not known. However, it is believed that it belongs to the myeloid precursor cell population.

Colony formation is dependent on the presence of cells producing the colony-stimulating factor (CSF). The mature granulocyte has for a long time been suggested as the major source of CSF in the peripheral blood (9, 20, 21). Recently however it has been shown that the CSF-producing cell in the peripheral blood is a mononuclear cell, most probably the monocyte (4, 6, 8).

CFC have also been demonstrated in bone marrow and peripheral blood from patients with acute leukemia (10, 17, 19, 21). Some authors have claimed that peripheral blood cells from patients with acute myeloid leukemia (AML) have a larger than normal colony-forming capacity (17, 19) while others have shown that a large number of patients lack CFC in the blood (16).

The presence of colony-stimulating cells, here for simplicity called CSC, has also been demonstrated in the peripheral blood from patients with leukemia. Most investigators have found that the peripheral blood cells of patients with AML fail to induce colony formation (18) or do so only when the patients are in remission (9). Others could not find such a correlation to the clinical state of the patient (15).

It has thus been demonstrated that certain patients with acute leukemia have either CFC, CSC or both, while others have none of these cells in their blood. In this work an attempt was made to find out whether the presence of CFC or CSC was of prognostic significance.

MATERIAL

Fifteen adult patients with leukemia—ten AML, two acute undifferentiated (AUL), one chronic myelocytic (CML) in blastic phase, one erythroleukemia and one regenerative anemia which later progressed to erythroleukemia—were investigated (Table I). The patient with regenerative anemia and later erythroleukemia was investigated in both phases of the disease. She was treated with oxymetholone and prednisolone in combination at the time of sampling, and no therapy with cytotoxic drugs has as yet been instituted. The patient with CML was treated with a combination of vincristine and prednisolone and irradiation of the spleen. All other patients with acute leukemia were treated with intermittent

Table 1 Initial data on 15 untreated leukemic patients including colony-forming and colony-stimulating ability in 10^4 peripheral white blood cells

Pat no	Sex	Age (y)	Diagnosis	Total WBC/mm ³	Blast cells (%)	Trombocytes/mm	No. of colonies/ 10^4 cells ^a	
							Colony forming ability	Colony-stimulating ability
1	♀	67	AML	56 000	97	10 000	0 0 0	0 0 0
2	♂	58	AML	48 000	40	70 000	0 0 0	0 0 0
3	♂	81	AML	161 000	91	44 000	0 0 0	0 0 0
4	♀	70	AML	22 000	90	52 000	0 0 0	0 3 2
5	♀	72	AML	96 000	84	62 000	8 7 11	0 0 0
6	♂	83	AML	51 000	86	10 000	3 7 5	0 0 0
7	♀	63	AML	5 000	81	69 000	5 3 6	3 2
8	♀	64	AML	2 600	3	67 000	2 1 1	2 2 3
9	♀	52	AML	19 600	85	39 000	19 18 16	6 6 4
10	♀	35	AML	4 500	53	134 000	1 2 2	6 3 3
11	♀	56	AUL	3 500	11	81 000	13 15 12	6 8 4
12	♂	60	AUL	16 400	10	3 000	13 10 14	0 0 0
13	♂	71	CML, blast phase	48 200	45	539 000	>370 >350 >350	4 4 4
14	♀	65	Erythro-leukemia	15 300 ^a	54	11 000	0 0 0	4 2 2
15	♀	32	Acute myeloid leukemia, later erythroleukemia	2 000	0	62 000	8 10 8	2 3 4

Healthy controls, mean

10

Healthy controls, range

1-55

Total no. of nucleated cells 39 myeloblasts, 15 erythroblasts. ^a Results from each of triplicate plates.

courses of the combination rubidomycin+cytosine arabinoside+prednisolone with or without pretreatment with l-asparaginase (11).

At the time of the first sampling for CFC and CSC the patients were untreated except for blood transfusions (nos 1, 2, 6, 8, 10, 12 and 14). All the following samples were drawn during the rest period 2-31 days (median 9) after a course of chemotherapy. Such follow-up investigations could be performed in 12 of the 15 patients. Four patients entered remission and are still alive in their first remission lasting various periods (Table IV). The other patients did not respond to therapy and have succumbed except for patient 15 who is alive but not in remission.

Sixteen healthy persons were investigated repeatedly and simultaneously for comparison (37 samples).

METHODS

Blood sampling. Venous blood, 5-10 ml, with 20 IU heparin/ml was collected from leukemic patients and healthy donors.

Separation of white blood cell (WBC) from heparinized blood was done as described by Böyum (11). Ten parts of a 33.9% Isopaque solution (Nyegard, Oslo, Norway) were mixed with 20 parts of a 6% Dextran 500 solution (Pharmacia, Uppsala, Sweden). One part of heparinized blood was layered over three parts of the Isopaque-Dextran solution and allowed to stand at room temperature. The WBC-rich plasma layer was pipetted

off when the RBC mass had passed the interphase between the two layers, which took about 20-30 min.

Culture technique. The method has been described by Robinson and Pike (21) and Robinson et al. (19) in detail and was used without modifications.

Feeder layer. The culture medium was a modified preparation of McCoy's 5 A medium to which 15% fetal calf serum had been added. The medium was mixed with boiled agar to give a final agar concentration of 0.5%. WBC obtained by Isopaque-Dextran sedimentation, after being carefully washed, were added to the mixture to a final concentration of 1×10^6 cells/ml. Aliquots of 1 ml of this mixture were then pipetted into 35 mm Falcon petri dishes and allowed to gel at room temperature before the overlay was added.

Overlay. Peripheral WBC were added to a mixture of modified McCoy's 5 A medium with 15% fetal calf serum and 0.3% agar to a final concentration of 1×10^6 cells/ml. One ml was then plated over the feeder layer and allowed to gel at room temperature. All cultures were set up in triplicate and incubated in a humidified incubator at 37°C and 10% CO₂ in air. Colonies in the overlay were counted in an inverted microscope (Olympus, Tokyo) after 14 days of incubation.

CFC in the peripheral blood from leukemic patients were studied by plating their WBC over a feeder layer of normal human peripheral WBC. CSC in the peripheral blood of the patients were studied by using leukemic WBC as feeder layer in cultures with normal human peripheral WBC in the overlay. Control cultures of

Table II Colony-forming and colony-stimulating ability in patients who could be sampled quantitatively during treatment

Pat. no.	Diagnosis	Sample time after diagnosis (d.)	Total WBC/mm ³	Blast cells (%)	No. of colonies/10 ⁵ cells	
					Colony-forming ability	Colony stimulating ability
4	AML	0	20 000	40	0 0 0	0 0 0
		13	3 000	0	0 0 0	0 0 0
		40	11 000	7	0 0 0	0 0 0
		0	22 700	90	0 0 0	0 3 2
		35	6 000	98	0 0 0	0 2
5	AML	48	1 600	56	0 0 0	1 2 2
		0	62 000	84	37 11	0 0 0
		9	7 700	84	3 2 1	0 0 0
6	AML	37	3 000	18	1 2 1	0 0 0
		0	51 000	86	3 7 5	0 0 0
		9	10 400	91	3 2 1	0 0 0
		37	12 000	94	1 1 1	0 0 0
8	AML	0	2 600	3	2 0 1	2 3
		7	1 200	0	18 16 16	4 3 2
		60	900	0	0 0 0	1 2 1
		120	4 200	0	2 1 2	1 1 1
		190	3 290	0	2 1 2	1 1 1
		215	2 900	0	1 1 1	1 0 2
		0	19 600	85	19 18 16	6 6 4
9	AML	10	1 800	51	6 4 7	7 5 5
		30	2 700	0	9 7 8	10 9 9
		70	4 100	0	1 1 1	0 1
		116	4 900	0	0 0 2	1 2 1
		190	3 500	0	1 0 1	Not done
		0	4 900	53	1 2	6 3 3
		20	900	0	0 0 0	13 18 15
11	AUL	102	4 500	0	6 5 5	5 1 4
		0	3 500	11	13 15 12	6 8 4
		28	680	0	0 0 0	2 1 2
		68	8 800	0	2 1 3	3 1 1
12	AUL	0	16 400	10	13 10 14	0 0 0
		8	2 400	32	23 21 22 4	7 6 8
		66	1 200	0	6 8 7	0 0 0
13	CML, blast phase	0	48 200	45	>350 >350 >350	4 4 4
		28	163 000	77	192 167 182	7 5 8
14	Erythro-leukemia	0	55 300*	39/19*	0 0 0	4 2 3
		32	17 500*	55/29*	0 0 0	27 31 37
		6	65 600*	69/9*	0 0 0	0 0 0
15	Aregenerative anaemia, later erythro-leukemia	0	2 000	0/0	3 10 8	3 4
		46	4 500	0/0	280 300 280	18 16 16
		105	13 900*	2/32*	8 6 8	Not done
		142	31 500*	0/94*	0 0 0	0 0 0

Total number of nucleated cells Myeloblasts/erythroblasts. Results from each of triplicate plates.

peripheral WBC from healthy subjects were set up at each experiment using cells from the same healthy individual both in the feeder layer and the overlay.

RESULTS

Colony-forming cells

Eight of the 15 patients with AML or AUL had CFC before treatment. No correlation could be found either to total WBC counts or to the per cent blast cells at the time of the sampling (Table II).

Peripheral WBC from healthy controls gave rise to a mean number of 10 colonies (range 1-35) (Table I). The patient with CML in blastic phase had a markedly increased number of CFC. The patient with erythroleukemia did not form colonies and the patient with aregenerative anaemia had an initial normal or high colony-forming ability (Table II) which however during the progression of the disease to erythroleukemia went down to 0 (Table II).

Table III. Morphology of colonies in different stages of the disease

RM=remission LI=large loose Lc=large compact, Sc=small compact, Nc=no colonies

Pat. no.	Diagnosis	Clinical state	Colony type
5	AML	Untreated No RM No RM	Lc Sc Sc
6	AML	Untreated No RM No RM	Lc Lc Sc
8	AML	Untreated No RM Partial RM Complete RM Complete RM Complete RM	Sc Sc Nc LI LI LI
9	AML	Untreated No RM Partial RM Complete RM Complete RM Complete RM	Lc Lc Sc Sc Sc Lc
10	AML	Untreated No RM Complete RM	Sc N LI
11	AUL	Untreated No RM Complete RM	LI Nc LI
12	AUL	Untreated No RM No RM	LI Sc Sc
13	CML, blast phase	Untreated No RM	LI LI
15	Aregenerative anemia, later erythro-leukemia	Untreated No RM No RM No RM	Sc LI LI and Sc Nc

The colony-forming ability generally persisted during treatment and disappeared only during severe leukopenia induced by cytotoxic drugs (patients 8 and 10 at a WBC count of 900/mm³ and patient 11 at 680/mm³ Table II). All four patients who entered remission belonged to the group with initial colony-forming ability.

Colony stimulating ability

Six of the 12 patients with AML or AUL had CSC in their peripheral blood. Such activity was also present in both erythroleukemia patients and in the patient with CML in the blastic phase. The average number of colonies induced by leukemic cells with colony-stimulating ability was however lower than

the number of colonies induced by normal feeder cells (Table I). All patients with AML or AUL who initially demonstrated this activity continued to do so during treatment. Only one patient who lacked stimulating ability initially acquired this ability after treatment. Both patients with erythroleukemia lost their stimulating ability during progression of the disease (Table II). All four patients who entered remission belonged to the group with colony-stimulating ability. Only two of the 12 AML or AUL patients with stimulating cells (nos 4 and 7) but all six without these cells died without remission.

Colony morphology (Table III)

The morphology of colonies after 14 days of *in vitro* incubation could be divided into three groups: large loose, large compact and small compact types. Peripheral WBC from healthy donors give rise to large colonies, usually of the large loose type but occasionally also of the large compact type. The small compact type colonies were composed of 50-170 cells with an average of 75 cells per colony; the large types of 300-1200 cells with an average of 700 cells per colony. In the patient material all types of colonies were represented at the time of the first sampling. The AML patients and the patient with aregenerative anemia had colonies of the compact type while the two patients with AUL and the patient with CML in the blast phase had the normal loose type of colonies. During treatment the morphology of the colonies remained unchanged or changed to any of the three types. All four patients who went into complete remission developed the large normal type of colonies during remission, three of the four had the large loose type and one the large compact type of colonies, while all three AML or AUL patients who could be sampled over a sufficient period and who did not enter remission had the abnormal small compact type of colonies at the end of their disease.

Colony-forming and colony-stimulating cells in relation to prognosis (Table IV)

The combined presence of CFC and CSC was found in all four patients with AML or AUL who entered remission. Of the remaining patients with these diagnoses only one had the same combination without entering remission. A similar combination was however found in the patient with CML in blastic crisis, who died, and in a patient with

Table IV Colony-forming and colony-stimulating cells in untreated patients and the clinical course of the disease

Pat. no	Diagnosis	Colony-forming ability	Colony-stimulating ability	Clinical course	
				Complete remission time (d)	Survival
1	AML	-	-	None	Died 8 d. after diagnosis
2	AML	-	-	None	Died 93 d. after diagnosis
3	AML	-	-	None	Died 17 d. after diagnosis
4	AML	-	+	None	Died 100 d. after diagnosis
5	AML	+	-	None	Died 51 d. after diagnosis
6	AML	+	-	None	Died 40 d. after diagnosis
7	AML	+	+	None	Died 70 d. after diagnosis
8	AML	+	+	210	Alive 770 d. after diagnosis
9	AML	+	+	166	Alive 223 d. after diagnosis
10	AML	+	+	205	Alive 256 d. after diagnosis
11	AUL	+	+	58	Alive 124 d. after diagnosis
12	AUL	+	-	None	Died 90 d. after diagnosis
13	CML, blast phase	++	+	None	Died 38 d. after diagnosis
14	Erythroleukemia	-	+	None	Died 89 d. after diagnosis
15	Aregenerative anemia, later erythroleukemia	+	+	None	Alive 173 d. after diagnosis of anemia, 57 d. after diagnosis of leukemia

+ = within normal limits, ++ = more than normal.

aregenerative anemia who later developed erythroleukemia and is alive but not in remission. In the overt leukemic phase of the latter patient the CSC found in the initial stage of the disease had disappeared. It thus appears that an initial combination of CFC and CSC may be a good prognostic sign in patients with AML or AUL.

DISCUSSION

Peripheral WBC from patients with acute leukemia were shown in this study as in other previous studies (16-19) to be able to form colonies in an *in vitro* agar culture system. A great variation in the morphological appearance of the colonies was also noted. Both the normal large and the abnormal small types of colonies previously observed in patients with acute leukemia (18) were found. Whether this variation in colony morphology can be explained by the proliferation of different cell clones normal and leukemic is still unclear. It has been convincingly shown using chromosome markers that leukemic cells are capable of forming colonies in agar (2, 5-14) but it is still debated whether normal cells as well may form colonies in cell cultures from untreated patients.

During treatment the morphology of the colonies either changed from the original appearance to any of the three patterns: small compact, large compact, large loose or remained unchanged. Although there was no clear correlation between the type

of change and the prognosis, all four patients who entered remission had the normal large type of colonies in this stage of the disease. This observation is in agreement with previously observed normalization of the colony type in remission (14).

Most of the patients in the present study formed colonies before institution of treatment, which is at variance with some other studies (9-16). It has also been reported that cells from patients with a high number of blast cells give rise to the greatest number of colonies (19). We could not find this type of correlation. During treatment there was generally no change in the colony-forming ability. Only during severe leukopenia was this ability lost. Thus cytotoxic drugs are capable of destroying the CFC in the peripheral blood, although these cells seem to be more resistant to therapy than most other cells. Such therapeutically induced disappearance of CFC occurred in three of the four patients entering remission and in all these three (nos. 8, 10, 11) large loose colonies reappeared.

In the patient with CML the number of colonies was very much increased, which is in agreement with previous findings by Moore et al. (16). In the blasto. state, however, we did not find any apparent decrease in the colony-forming capacity previously noted by the same authors. The patient with preleukemic aregenerative anemia had initially normal colony-forming ability in contrast to some reports of abnormal clones in preleukemia (6, 22).

However, after progression to erythroleukemia no colonies were formed and this pattern was similar to that found in the second case of erythroleukemia.

None of the six patients in our study who initially lacked cells with colony-stimulating ability entered remission while all four who did so had cells with colony-stimulating activity. During treatment there was generally no change in the ability to stimulate colony formation. Only one patient with initial inability to stimulate colony formation had a transient change to colony-stimulating activity during treatment.

In this study the presence of both CFC and CSC was found in all four patients who entered remission. Only one AML patient with this combination did not enter remission. It is not possible from our investigation to state that this is a requirement for remission. However, it seems intriguing to speculate that the presence of these two cell types found also in normal persons may be of prognostic importance. The patient with CML in the blastic phase as well as the patient with regenerative anemia, both had colony-forming and colony-stimulating ability. Here a combination of CFC and CSC was not of prognostic value. However, the progression of these diseases may have a different pathogenesis than that of ML and AUL.

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DEFECTIVE DIFFERENTIATION OF MEGAKARYOCYTES IN ACUTE MYELOID LEUKEMIA

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Abstract 1 patient with acute myeloid leukemia chromosome analysis, ring banding techniques revealed as karyosome 17 in granulopoietic and erythropoietic precursors as well as in polyploid cells, probably megakaryocytes. An abundant number of megakaryocytes in the bone marrow permitted characterization of the ploidy levels of these cells. It is demonstrated that most megakaryocytes were diploid and that polyploidy was not normally developed. The results indicate defective differentiation of the megakaryocytes and/or their precursors analogous to what has previously been demonstrated in the myeloblasts of acute leukemia.

An increasing amount of data indicate that leukemic blast cells have a low proliferative activity and reduced ability to differentiate (9-10). A delayed maturation of these cells has been demonstrated even in the preleukemic phase (6). Arough Jensen and Killmann (11, 12) have presented evidence that erythroblasts may likewise be of leukemic origin in acute myeloid leukemia (AML), and it has been suggested that the proliferative activity of the erythroblasts is decreased (7, 17). Less is known about the proliferation and differentiation of megakaryocytes in AML, although thrombocytopoiesis is obviously always affected in acute leukemia.

In the present report of a patient with acute leukemia chromosome studies indicated an abnormally low degree of polyploidy in the megakaryocytes pointing to a reduced differentiation of these cells in line with what has been reported in leukemic blast cells.

CASE REPORT

The patient is 78-year-old female with increasing fatigue and tendency to bruising and epistaxis over the past 3-4 months. On admission Hb was 7.1 g/

100 ml, RBC 2.6 $\text{mill}.$, Hct 22.1%, MCV 96 μ^2 and MCHC 34 g/100 ml. The WBC count was 700 and a differential count showed 24% neutrophils, 34% lymphocytes, 30% monocytes, 2% eosinophils and 3% basophils. No definite blast cells were seen but 7% of the cells were classified as small megakaryocytes. The thrombocyte count was 75 000. The bone marrow findings are described below (see Results). Serum iron was 130 $\mu\text{g}/100$ ml and transferrin 260 $\mu\text{g}/100$ ml. Serum B $_2$ 3.90 $\mu\text{g}/\text{ml}$ and folic acid in whole blood was also normal 27 $\mu\text{g}/\text{ml}$. Serum lysocytase was normal and the urine contained no detectable lysocytase. An abdominal X-ray revealed no splenomegaly.

During the first two weeks after admission the Hb concentration fell to 5.4 g/100 ml and transfusions were given. The patient has hitherto been followed for 6 months and repeated blood transfusions have been required. WBC have remained low about 1 400-2 000 and the thrombocyte count has fallen to about 25 000. There has been successive increase in the proportion of blast cells in the bone marrow from 11% to 40%.

MATERIAL AND METHODS

The investigations described below were performed immediately after the first admission.

Bone marrow smears were stained with May-Grunwald-Giemsa and differential count of 1 000 cells was performed. One thousand mitotic cells were examined and the proportion of mitotic figures assigned to megakaryocytes was determined.

Chromosome preparations. A bone marrow aspirate was collected in heparinized syringe and immediately incubated at 37°C in Basal Medium Eagle containing 0.1 $\mu\text{g}/\text{ml}$ Colcemid®. After 1 hour the cells were rinsed twice in Hank BSS, transferred to 0.075 M KCl for 20 min as hypotonic treatment and subsequently fixed in several changes of methanol:acetic acid 3:1. Air-dry spreads were prepared and stained with Giemsa according to the method described by Seabright (21) with slight modifications. The chromosomes were classified according to criteria given by the Paris Conference (1971) (16).



Fig. 1 Karyotype of a diploid bone marrow cell stained by a G-banding technique showing one chromosome 17 replaced by one isochromosome 17 (arrow).

RESULTS

The bone marrow smears were very rich in cells with a myeloid:erythroid ratio of 3:1. There was a striking predominance of immature granulopoietic cells and 11.0% of the bone marrow cells were assessed as myeloblasts. In addition an accumulation of megakaryocytes 7.8% was noted. Most of these cells were immature with a basophilic non-granulated cytoplasm. Within the erythropoietic pool a considerable number of cells with a megakaryoblastoid appearance were noted. Of the mitotic figures 57.6% were found in granulopoietic precursor cells, 39.7% in erythroblasts and 2.5% in megakaryocytes.

Chromosome analyses. The scanning of 3000 metaphases for polyploidy resulted in the finding of 7 octaploid and 21 tetraploid cells. The chromosomes of 100 diploid and 2 tetraploid cells were counted. All the diploid cells had 46 chromosomes and both tetraploid 92. The two tetraploid cells and 35 of the diploid cells were karyotyped. All diploid cells analysed showed the same pseudodiploid karyotype: one chromosome 17 was replaced by one metacentric marker chromosome of C group size. The Giemsa banding pattern of all cells analysed indicated that the metacentric marker was an isochromosome for the long arm of a chromosome 17 (Fig. 1). The two tetraploid cells

showed the exact double pseudodiploid karyotype (Fig. 2).

DISCUSSION

Several clinical and laboratory data on the patient are highly suggestive of a malignant hematologic disease. Anemia, thrombocytopenia and leukopenia associated with a hypercellular bone marrow dominated by immature granulopoietic cells and with a high proportion of myeloblasts are consistent with the diagnosis of acute leukemia. The constant cytogenetic abnormality including a conspicuous marker chromosome in all bone marrow cells also provided strong evidence in favor of the diagnosis of leukemia. There was also an accumulation of immature megakaryocytes in the bone marrow similar to what has previously been described as acute aleukemic megakaryocytic leukemia (1, 18) and in a case of refractory anemia highly suggestive of aleukemic ANL (2).

Chromosome abnormalities are present in about 50% of acute leukemias (20) and it has been demonstrated that the karyotypic abnormalities may be common to granulopoietic and erythropoietic cells (11, 12). The results in our patient confirm that granulopoietic precursors and erythroblasts may have a common chromosome aberration since in



Fig 2 Metaphase plate of tetraploid bone marrow cell stained by G-banding technique. The two isochromosomes 17 are indicated by arrows.

all bone marrow metaphases analysed by banding technique a marker chromosome—iso chromosome 17—was found to replace a normal chromosome 17.

The abundance of megakaryocytes in the bone marrow of the present case offered a relatively unique opportunity to study these cells. It seems well established that cells recognizable as megakaryocytes in the bone marrow are normally polyploid (13) and that 70-90% are cells with 16-ploidy or more (8-15). It has been demonstrated that DNA synthesis and nuclear replication occur only in the most immature megakaryocytes (3-5). In our patient 99% of the metaphases were diploid and only 0.8% were polyploid. Thus the percentage of polyploid metaphases was only one third of that expected from the analyses of the bone marrow smears. We therefore conclude that a large proportion of the megakaryocytes with mitotic figures were ≥ 4 cells. Furthermore 1 of 11 polyploid metaphases found in the chromosome preparations were tetraploid cells (8.5%) and only

two octaploid cells were noted. No cells with higher ploidy level were seen. Assuming that the polyploid mitotic figures observed belonged to the most immature megakaryocytes, these results are consistent with an abnormally low degree of ploidy in these cells, since Odell and Jackson (13) found 89% with ploidy values higher than $4x$ in immature megakaryocytes. No metaphase with $16x$ or more was found in the bone marrow of our patient, which further emphasizes that polyploidy was not normally developed in her megakaryocytes.

It has been suggested that individual megakaryocytes may reach full maturity and shed thrombocytes at different ploidy levels (4-14) and that the size of the mature megakaryocyte is related to the degree of polyploidy (14). Mature megakaryocytes with low ploidy values may therefore produce relatively few platelets. The finding of a low degree of polyploidy of the megakaryocytes in raises the question whether at least in part due to a reduced polyploidy. Despite the presence of

penia Saarni (19) found normal or increased numbers of megakaryocytes in the bone marrow of most patients with acute myelomonocytic leukemia. The degree of ploidy of the megakaryocytes in acute leukemic conditions with low platelet counts may therefore deserve further investigation in order to clarify the mechanism causing thrombocytopenia.

Ebbe (4) and Odell and Jackson (14) have proposed models for megakaryocytopoiesis in the rat. The evidence favors the existence of a morphologically unrecognized polyploid precursor pool that feeds the megakaryocytic compartment. The precursor compartment is composed of cells capable of nuclear replication but not of cell division. Assuming that a similar compartment exists in man the finding of immature megakaryocytes with a low ploidy in our patient may indicate that replication was blocked before the cells had entered the recognizable megakaryocytic compartment.

Our results indicate a common chromosome abnormality in granulopoietic erythropoietic and megakaryocytic cells suggesting a clonal origin of all these types of cells in the present case. As mentioned initially several data argue in favor of a reduced capacity of the myeloblasts to differentiate in AML, and a similar defect is probably in the erythroblasts. The finding of an unusually low degree of polyploidy in the megakaryocytes of our patient indicates that the differentiation of megakaryocytes or their precursors may be blocked in a similar way in acute leukemia.

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IRON INTOXICATION IN TWO ADULT PATIENTS

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Abstract Iron intoxication in adults is very rare. Only a few cases are reported in the literature. This paper describes two successfully treated adult patients, one without complications and the other with circulatory failure hepatic involvement and hemolysis. The current opinions on pathogenesis and therapy are reviewed.

Acute poisoning with iron compounds is a common problem in clinical pediatrics. Some 2000 cases are reported annually from the United States (13). Formerly there was a high incidence of mortality (50%) but since the introduction of desferrioxamine a chelating agent promoting the urinary excretion of iron the prognosis has improved.

In adults iron intoxication is an unusual clinical problem and the iron compounds are mostly ingested with the intention of committing suicide. Only a few cases have been reported in the literature (4, 5, 10, 13). In the present report two cases of acute iron intoxication, one without complications and the other with hepatic involvement and hemolysis, which responded successfully to treatment will be described.

CASE REPORTS

Case 1

A 19-year-old woman who had immigrated to Sweden 4 years ago and had difficulties in her adaptation at work and conflict at home was admitted to the hospital 2 1/2 hours after ingesting 60 tablets of ferrous succinate corresponding to 2 g Fe^{++} or 42 mg Fe^{++}/kg b.wt.

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On admission the patient was drowsy, felt unwell and had epigastric pain. She had tachycardia (120 beats/min), BP 120/90 mmHg.

Immediately after admission vomiting was elicited. One hour later she was given 1 g Desferal (desferrioxamine) i.m. and then the same amount 3 hours later. During the first 24 hours she was kept in the Intensive Care Unit. Except for epigastric pains no signs of complication were noted.

Laboratory investigations: Initial Hb 1.5 g/100 ml, Hct 40%, Plasma iron 350 $\mu g/100$ ml, TIBC 310 $\mu g/100$ ml, i.e. 100% saturation (Fig. 1). WBC 6400/mm³, differential count normal. Urine sediment normal. Serum electrolytes and liver tests showed normal values. The variation in plasma iron concentration and the amount excreted via the urine are shown in Fig. 1. Stools had traces of blood one day after admission. One week after admission the patient left the hospital feeling completely well. An X-ray of the stomach 3 weeks later was normal.

Case 2

The patient, a 26-year-old woman, mother of three children, was admitted to hospital after ingesting 150 tablets of ferrous succinate, corresponding to 11 g Fe^{++} or 180 mg Fe^{++}/kg b.wt. She had had 1 abortion about 6 months earlier.

The tablets were ingested during 3 hours prior to admission. On admission the patient was dry, warm and comatose, but felt unwell. BP 190/110 mmHg.

Treatment with desferrioxamine 1 g i.m. was initiated 30 min after admission. When ventricular tube was inserted, profuse vomiting was elicited. The outputs soon became mixed with blood. Within few hours the patient became hypovolemic with tachycardia and falling BP and became pale and weak.

During the first 24 hours the patient was kept in the Intensive Care Unit. Partial blood exchange was performed, she was given 700 ml blood, and also fresh frozen plasma. An attempt was made to keep the diuresis at 50 ml/hour and she was given 500 ml mannitol (15%). On the first day the patient was given desferrioxamine 1 g i.m. and 5 g per os via the ventricular tube. On the second day she received 2.5 g

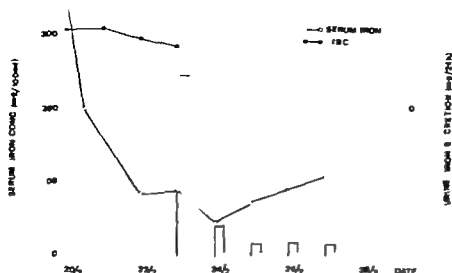


Fig 1 Serum iron, total iron binding capacity and urinary iron excretion in case 1

L. and 5 g per os in the tube and after that 1 g \times 4 i.m. during one week.

Laboratory investigations. Initial Hb 13.0 g/100 ml (4 h later 16.6 g/100 ml) Hct 37% (55%) Plasma iron 640 μ g/100 ml (maximum 7 h later 1400 μ g/100 ml). TIBC 597 μ g/100 ml *i.e.* 100% saturation (Fig. 1) WBC 19000/mm differential count normal. Bicarbonate 4 mEq/l. Na 142 mEq/l. K 3.9 mEq/l. Cl 110 mEq/l. Urine sediment, many red corpuscles.

In the first 4 hours she felt sick and vomited five times. The total volume of fluid administered was 4 l and the fluid loss 5.3 l. The volume of the vomitus was estimated to be about 6 l. Initially the CVP was very low, around and below zero. Systolic BP 140–100–120 mmHg. Temperature increased to maximum of 38 °C after 1 hour, and the patient remained

subfebrile for one week. After 24 hours she was allowed to drink, and she felt well from the 6th day.

After days she became icteric and polyuric (Fig. 3). She had 48 hours after admission a diuresis of 875 ml in one hour and during the 3rd 24-hour period of 8800 ml osmolality 97 mosmol/l. The liver tests showed pathological values (Table I) culminating on the 2nd day.

Hemolysis was indicated by the observation of free Hb in plasma and urine (Fig. 4) depletion of haaptoglobin (Fig. 5) and slight thrombocyte depression. A slight hypokalaemia was compensated. Sternal puncture was performed on the 4th and 11th days, the marrow showing no iron in the reticuloen cells.

The patient was checked one month after admission. She felt well and the results of routine laboratory tests were normal.

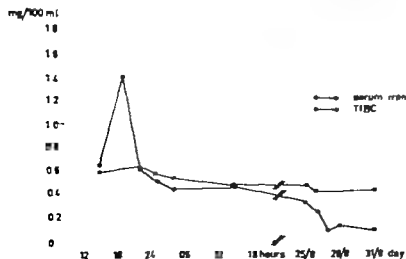


Fig 2 Serum iron and total iron binding capacity in case 2.

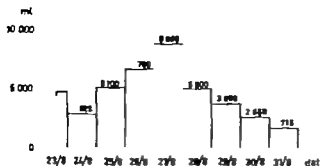


Fig 3 Urine volume (ml/24 h) osmolality (mosmol/l) and iron excretion (mg/24 h) in case 1.

DISCUSSION

Iron intoxication is an unusual clinical problem in adults but it is important to know how to handle such cases because there is a high mortality rate if adequate therapy is not instituted immediately.

The pathogenesis of acute iron poisoning is still controversial. When ferrous compounds are given orally to different experimental animals (8-11) damage in the gastrointestinal tract has been demonstrated with small hemorrhagic spots and some edema in the stomach and more severe changes in the intestine especially the duodenum, with large hemorrhages, shrinkage of the villi and severe necrosis of the mucosa. The same findings have been encountered at autopsy in patients—children and adults—dying after ingestion of such iron compounds (4-6).

These changes in the intestine probably due to local irritation by the ferrous compounds may explain the absorption. It is possible that the capil-

laries are opened due to damage to the mucosa and that the iron can diffuse directly into the blood stream. The absorption of iron through the apo-feritin mechanism is probably minimal. Thus the main etiology of the iron absorption may be the capillary damage although there are some reports that thrombotizing of intestinal vessels occurs very rapidly and this mechanism would be a limiting factor (4). In our two patients there was evidence of intestinal bleeding—positive test for blood in the stools of the first patient and hematemesis in the second.

Reports of damage to other organs are rare. Postmortem examination of a woman who died after oral ingestion of iron (4) showed bleeding in the lungs and kidneys. Microscopic examination showed fatty degeneration of the heart, the liver and the epithelial cells of the tubules of the kidneys. Our second patient showed evidence of liver damage—elevated S-GOT, S-GPT and bilirubin. She also showed signs of hemolysis as judged

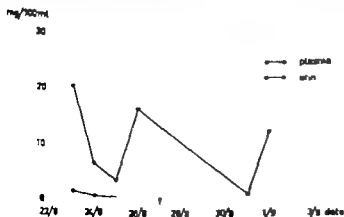


Fig 4 Free Hb in plasma and urine in case 2.

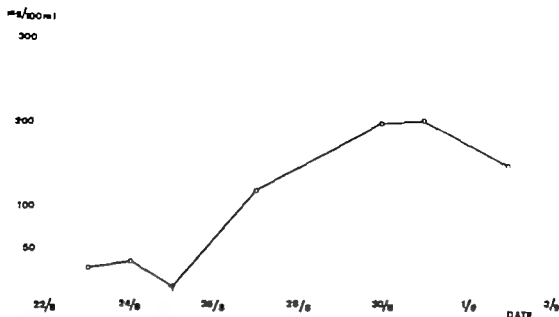


Fig 5 Serum haptoglobin concentration in case 1.

from free Hb in plasma and urine haptoglobin consumption moderate thrombocytopenia and elevated unconjugated bilirubin. This complication has not, as far as we know been reported in the literature. The occurrence of hemolysis in this patient is difficult to interpret but hypothetically there might be two mechanisms either damage to the membranes of circulating red blood cells or an interference with different enzyme systems similar to that which can be seen after copper and lead poisoning.

Hemodynamic alterations are one of the major problems in acute iron intoxication which may cause irreversible shock and death. Studies on dogs (14) one hour after administration of a lethal dose of ferrous sulphate into the intestine showed a sharp decline in cardiac output a smaller reduc-

tion of arterial BP and a marked elevation of peripheral resistance. Only minimal reduction in total blood volume was seen but the plasma volume was significantly reduced. Thus early correction of plasma and blood volume is important to prevent irreversible shock and to maintain renal function to permit excretion of sufficient quantities of iron.

Our second patient showed clear evidence of hypovolemic shock, which appeared a few hours after admission. She was treated with blood and plasma transfusions and attempts were made to keep her diuresis at about 50 ml/hour. The explanation of the hemodynamic alterations is still obscure and several mechanisms have been suggested. One is that bacterial toxins enter the blood through the damaged intestinal mucosae another

Table I Liver function tests in case 2

	Date						
	24/8	25/8	26/8	27/8	30/8	2/9	9/9
Total bilirubin (mg/100 ml)	13.8	17.2	13.6	5.6	2.2		0.5
Bilirubin conj.	7.7	12.0	10.8	3.9	1.4		0.6
Alkaline phosphatase	5	8		8			
5-GOT	34	421	167		26	3	17
5-GPT	31	495	489		97	68	23
LDH						236	

that unbound plasma ionic iron has a direct action on the cardiovascular system and promotes the collapse (2, 3).

Metabolic acidosis is often present together with cardiovascular collapse. The mechanism is not completely clear. It is reported that the metabolic disturbances start before the circulatory failure. It is assumed that hydrogen ion production results from conversion of ferrous to ferric iron in blood or that iron interferes with enzymes in Krebs cycle. After hemodynamic alterations with diminished cardiac output the acidosis is accentuated by tissue hypoxia and other metabolic disturbances (12).

Fever and leucocytosis are also noted in iron poisoning, probably due to tissue damage of different organs. Our second patient had fever for almost a whole week and leucocytosis ($19000/\text{mm}^3$) and evidently she had damage of the intestinal mucosa and parenchyma cells of the liver.

The most serious symptoms shock and/or coma, seem to be related to the plasma iron level. Below $500 \mu\text{g}/100 \text{ ml}$ only 8% develop this picture but when the concentration exceeds $500 \mu\text{g}/100 \text{ ml}$ it is more common (37%). When the concentration exceeds $1000 \mu\text{g}/100 \text{ ml}$ 70% of the patients have manifest hypovolemic shock (13). It should however be emphasized that even patients with plasma iron concentration below $500 \mu\text{g}/100 \text{ ml}$ can develop life-threatening shock (13, 15).

The clinical features of iron intoxication can be divided into 4 stages (1). During the first 6 hours there are different gastrointestinal symptoms such as vomiting, diarrhea, melena, hematemesis and epigastric pain, and during this time hypovolemic shock can develop. Then there may be a period of clinical improvement (6-24 h) but the patient may still be severely ill and during the next 48 hours metabolic acidosis, convulsions, coma, hepatic impairment and bleeding problems may arise. Several weeks later intestinal obstruction secondary to scarring can be encountered. Neither of our patients showed this late complication.

Once the diagnosis of an overdose of iron salt is suspected therapy should be started immediately. There is no time to wait for the serum iron level and furthermore the serum iron level does not always reflect the severity of the poisoning.

Vomiting should be provoked at once. Desferrioxamine treatment should be started as soon as possible (7, 9, 13). Desferrioxamine a potent,

specific iron-chelating agent, can bind iron in the gastrointestinal tract and the complex is poorly absorbed and therefore initially 5-10 g should be given orally. Furthermore to bind the circulating iron 1-2 g every 3rd-12th hour should be administered intramuscularly to patients not in shock and intravenously at a rate not exceeding 15 mg/kg/h to patients in shock or coma. The administration should continue for up to 3 or 3 days depending on the severity of the poisoning. In parallel with this specific therapy the patient should be monitored for shock symptoms, electrolyte disturbances and acidosis. The urinary output should be followed carefully and if necessary forced diuresis may be provoked so that the kidneys can excrete sufficient amounts of iron. If the serum iron is too high exchange transfusion can be performed initially (11).

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IODINE INDUCED TOXIC DIFFUSE GOITRE

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Abstract. A report is given of iodine-induced hyperthyroidism and goitre in a patient who ingested seaweed tablets for over two years and lived in an area of iodine sufficiency. There was spontaneous remission of both hyperthyroidism and goitre three months after withdrawal of excess iodine. This case shows that neither pre-existing iodine deficiency nor goitre are necessary for development of iodine-induced hyperthyroidism and that combination of iodine-induced thyrotoxicosis and goitre can occur. Various theories for the pathogenesis of iodine-induced hyperthyroidism are discussed briefly.

Hyperthyroidism is a rare complication of iodine treatment for goitre. This disease is often called Jod-Basedow, although first described by Coindet in 1821 (2). Iodine-induced hyperthyroidism usually occurs in goitrous patients or in persons living in iodine-deficient areas. An increased incidence of hyperthyroidism was observed in the United States in the 1970s after introduction of iodine prophylaxis (10-15). Recently Stewart et al. (20) found that iodinated bread in the iodine-poor area of Tasmania more than doubled the incidence of hyperthyroidism. Large amounts of iodine have been found to provoke hyperthyroidism in endemic areas (4) and in patients with autonomous thyroid nodules (5). Some patients with non-toxic nodular goitre living in areas of iodine sufficiency also became hyperthyroid during administration of pharmacological doses of iodine (18-21).

The present paper reports iodine-induced toxic diffuse goitre in a patient who ingested seaweed tablets rich in iodine for over two years. The patient was born and lived in an area of iodine sufficiency. There was complete spontaneous remission of both hyperthyroidism and goitre after withdrawal of excess iodine.

CASE REPORT

The patient is a 38-year-old female. All her four sons have suffered from goitre, toxic or toxic. The patient had urinary tract infection eight years ago and has also suffered from chronic constipation for several years. In 1969 she started to ingest seaweed tablets made in Norway in an attempt to cure her constipation. Seaweed tablets have been on the market in this country for about ten years. In other respects her diet seems to have been well balanced. She claims that the seaweed tablets improved her bowel function and she therefore continued to take four tablets a day regularly.

In the summer of 1972 nervousness and tremors started to trouble her. She also observed increased sweating and tremor of the hands and was easily fatigued. These symptoms disappeared spontaneously after two months but recurred more intensely in Jan. 1973 after an acute respiratory infection. She still continued taking the seaweed tablets until Feb. 2, when she noticed that she had goitre. She consulted general practitioner but did not mention her seaweed regimen. Diffuse enlargement of the thyroid gland and pulse rate of 90/min were found. The goitre was firm, non-tender and clearly visible. There were no eye signs. Both serum protein-bound iodine (PBI) and triiodothyronine *in vitro* uptake test showed highly elevated values (Table 1).

She was sent to hospital for thyroidectomy. When found to suffer from hyperthyroidism she was referred to the Fourth Department of Medicine, University of Helsinki, to obtain anti-thyroid medication before the operation. This caused a one-month delay in treatment during which time her condition improved. On March 3 her hyperthyroidism was only of moderate severity and the goitre had decreased to twice the normal size as judged by palpation. Serum thyroxine determined with modification of the Murphy method (12) was elevated and so was the serum free thyroxine concentration (14). PBI had decreased to 0.90 $\mu\text{mol/l}$. No circulating thyroid antibodies were detected by the standard tanned red cell (anti-thyroglobulin) and complement fixation (anti-microsomal) techniques. There was no long-acting thyroid stimulator activity in the blood. ESR and serum

Congress Announcement

La Société Européenne de Cardiologie et la Société Française de Cardiologie organisent à Paris du 17 au 20 Mars 1975 inclus un cours en langue française sur les bases scientifiques de la physiopathologie cardiovasculaire.

Les thèmes en sont. Bases moléculaires de la contraction cardiaque. La paroi artérielle normale et pathologique. Bases physiopathologiques de l'hypertension artérielle et de son traitement. Fonction sinusale et activité auriculaire. Transport

et utilisation de l'oxygène. Ce cours est destiné à de jeunes médecins intéressés aux développements de la cardiologie fondamentale et clinique. Une connaissance précise de la langue française est nécessaire. Le nombre de places est limité.

Les demandes d'inscription sont reçues jusqu'au 31 Octobre 1974 par Docteur René Gourgon Hôpital Lariboisière 2 rue Ambroise Paré, 754 75 Paris Cedex 10

EDITORIAL

Medical oncology — Is it needed as a specialty?

In recent years medicine has developed along new lines and we are realizing that many malignant tumours are more wide-spread systemic than we thought a decade ago. Hematology has taught us interesting lessons from the field of lymphoma, plasmacytoma and CML. In these conditions many physicians agree that eradication therapies sterilisans magna in the old sense as used by Ehrlich for *Salvianum* in syphilis is no longer the immediate goal. Our aim is to keep the malignant cells down. In the last decade we have become a little more optimistic regarding real healing of acute blast cell leukemia in the younger patients. It is perhaps correct to talk about eradication of the last cell when healing has lasted for many years even in the no longer treated patient.

We regard repopulation of the body organs by mobile elements from the bone marrow as normal ever since Jacobson made his first classical experiments with shielding of the spleen during otherwise lethal irradiation. Thus he saved the animals. Schoenheimer wrote about the dynamic state of body constituents meaning molecules always changing in the metabolic flux. We may also speak about the mobility, the flux of seemingly fixed cells including microscopic tumour cells. Such a concept means that not only the knife but also cytostatic drugs, antibodies, enzymes, cell-seeking isotopes, etc. will have an ever increasing importance for the understanding of cancer and the war against the moving cancer cell. If we believe that eradication of the last cancer cell is something for Utopia, it may be that we can still help the patient to live with his cancer. One possibility might be to learn about the neutralization of the toxic substances that cause cachexia by poisoning metabolism. This may be brought about in many ways but not with knives nor with rays. Competitive action of synthetic drugs or specific antibodies directed against the presumed

toxins are possibilities that must be tested systematically.

Another line in modern oncology follows the leads that we may find through the study of the metabolic activities of cancer cells.

Everybody nowadays agrees to the fact that the dynamic metabolic outlook on disease has been one of the cornerstones of medical progress in recent years. If we were asked to give one single phrase describing what we mean by disease I would say: disturbed homeostasis. It is our first duty as clinicians to restore the balance that has been disturbed and there are many ways to do this by correcting metabolic processes.

Clinicians have become increasingly aware of the fact that carcinoma patients may suffer more acutely from symptoms that are described as paraneoplastic than from the direct action of the tumour. The most intriguing forms of paraneoplasia are connected with what we call 1) ectopic hormone formation by cancer cells, i.e. the tumour produces polypeptides that are metabolically active. There are other paraneoplastic phenomena that are much more obscure as far as the mechanism is concerned such as 2) neurological or autoimmune symptoms occurring in cancer patients. 3) Another group of symptoms may be explained as a return to fetal metabolism. 4) Finally there is the diagnostically very important group of symptoms caused by metabolic products from the normal cells that have become malignant. In this case the situation develops from normal "topic" production of active substances.

Common to all these phenomena is the fact that the symptoms should be reversible when the tumour is removed. This is the crucial proof of the role of the cancer cells.

What we now call paraneoplasia was first intuitively suspected by Fuller Albright, when he saw a

patient, who had severe hypercalcaemia but no signs of bone metastases from a renal carcinoma. The first to collect a convincing clinical material were Pimpton and Gellhorn who described no less than 10 patients with hypercalcaemia and malignancy. In one patient the hypercalcaemia disappeared after resection of the tumour and recurred with the metastases. Bone metastases were not present and the parathyroid glands were normal at the post-mortem. This severe clinical condition, that rapidly leads to death if not recognized, is often easy to treat and therefore quite important from a practical point of view. It has been found that it is not rare. The cause must be formation by the malignant cells of a substance identical with or similar in action to parathormone. For a very recent excellent discussion of this problem the reader might consult the June number of the *American Journal of Medicine*.

Other examples of a similar kind are the development of polycythemia by not only renal but also by other tumours. This is thought to be caused by the production of erythropoietin that is normally produced in the kidney. A large number of patients who have been cured from this complication after resection of a uterine fibroma has now been published, one patient also from Sweden.

Cushing's disease, often with strong pigmentation, is said to be more common as the result of ectopic ACTH (+MSH) production by carcinomas than it is as a primary malady. Severe hypotension often leads to marked adynamia that may be reversible. Curious endocrine symptoms may be because of the ectopic production of gonadotropins etc.

It seems as if all these symptoms were pretty heterogeneous. There are however certain clearcut features common to them all. They are all caused by active substances (hormones) starting endocrine syndromes that are recognizable clinically. The hormones are all polypeptides biochemically and the theory seems pretty convincing that the increased production is caused by derepression of polypeptide forming templates. This would mean that there is derepression of protein forming templates (RNA-DNA) in the cancer cell.

Are there other instances of sleeping templates for protein synthesis that may be derepressed in carcinogenesis? A recent finding seems most interesting. In 1944 Kai Pedersen in Uppsala discovered a new serum protein in the blood of newborn calves.

It was called *fetuin* and at that time it could not be demonstrated in human blood. It has now been established that it occurs in the serum of patients with malignant hepatoma. In some such sera it is quite abundant and may even be observed directly as an α_1 -fraction. In many sera from hepatoma patients it is not found and it has been established that it is present in animals with hepatoma caused by some carcinogens but not in tumours caused by others. Fetuin is definitely formed in the liver. Its presence in hepatoma is therefore not due to ectopic production but rather to a chronological error – an awakening of a template that became repressed at birth.

Recently a patient has been observed who had bronchial carcinoma and an anaemia characterized by a very marked synthesis of fetal hemoglobin (>35%). It is known that pregnant women have a higher hemoglobin F content than they have when non-pregnant. This points to the fact that they may respond to a factor from the fetus that derepresses their dormant template for hemoglobin F synthesis.

What is important in all these conditions is that such paraneoplastic symptoms should lead to a thorough examination regarding carcinoma, that even in inoperable conditions the paraneoplastic symptoms may be responsible for most of the patient's subjective complaints. Hypercalcaemia and hypokalaemia may be treated symptomatically even if the tumour cannot be eradicated and the patient thus be restored to a much happier life for some time.

It has long been known that certain clinical symptoms may be connected with special tumours. Hypertension in pheochromocytoma, flushes in carcinoids, acanthosis nigricans in tumours of the gastrointestinal tract are such examples that may lead to correct diagnosis if correctly interpreted. In the first two examples metabolic investigations have given us biochemical methods to make objective diagnoses. Such examples are steadily increasing in number and importance. Also for the checking of therapeutic results such parameters of metabolic type are important (cf. also acid phosphatases in prostatic carcinoma).

For the recognition of all these symptoms and of a number of others that will be discovered in rapid succession it is necessary that the oncologist has a good training in internal medicine, especially in hematology, endocrinology and metabolism.

Also treatment with an ever-increasing number

of very active — sometimes too toxic — antineoplastic drugs must be managed by well-trained internists. There is a wide-spread concern in present day society that modern drugs are too dangerous. It may be important for us as doctors to remember the Latin lines from the Aeneid that describes the feelings of the Trojans regarding the Trojan horse. They feared the Greeks even when they were

bringing gifts. We may well remember that "Greeks may be substituted by doctors — *Tl-meo medicos in dona ferentes*."

Well-trained specialized oncologists will diminish that fear and be able to help in conquering the most dreaded of all diseases — the malignant tumour in the widest sense of the word.

Jan G Waldenström

BOOK REVIEWS

Paraneoplastic syndromes. Annals of the New York Academy of Sciences. Volume 230 577 pp. March 1974

This symposium has resulted in a volume of nearly 600 pages. The attempt to cover the whole field is on the whole very satisfactory even if some interesting conditions are not mentioned, such as mastocytosis and hepatomas with cutaneous porphyria. Many topics are treated several times in different chapters. This is partly explained by the fact that the number of active participants in this conference was very large. These circumstances make it difficult to find a certain subject as there is no index. The bibliography of American literature is excellent and the book is on the whole a gold mine of information. With very few exceptions the speakers come from the United States. European contributions to the field are often not discussed even when they are quite important.

The first part treats generalized perturbations of host physiology with such subtitles as fever, cachexia, immunology and some general metabolic disturbances. It is disappointing to find how little factual knowledge we have about these important subjects but it is also a challenge to new work. The next part contains some admirable presentations regarding the problems of ectopic hormone formation. There are further excellent chapters on erythrocytosis, granulocytosis and disturbances seen in conditions with lymphocytic or plasma-cellular proliferation. This last subject is of course specially dear to the reviewer's heart and I may well say that the presentation is admirable, both full of facts and of ideas for future research.

It is difficult to choose some special topics among the many excellent presentations of other important problems. The discussion of "our evolving understanding of the leucoencephalopathy" by Richardson, who has been eminently active himself and through his group (Åström) to prove that this condition is not really direct paraneoplasia but specific viral disease.

The two chapters on renal problems are by Chisholm from Hammer-smith Hospital, London and one

by Fickman and Bethune. Southern California School of Medicine, Los Angeles, make excellent reading and could hardly present the diverse and difficult facts in a better way. This is also true of Waldman's chapter on protein losing enteropathies that is written by person, who is really master of his subject.

It is clear that all chapters cannot be equally good. Perhaps the most disappointing is the presentation of paraneoplastic symptoms visible on the surface — e. g. skin and appendages. This fascinating subjects treated in such way that the reader gets confused picture of cause and effect. The author has obviously not grasped the real meaning of paraneoplasia.

The biological mechanisms behind all these clinical findings is of course the most intriguing problem in this volume. They are discussed on the last 40 pages in a very stimulating way. The reviewer has always regarded Gordon's experiments with enucleated and re-nucleated *Xenopus* eggs as an excellent argument regarding derepression of reactivated (sleeping) chromosomes in the genome. Paraneoplasia as an example of specific derepression in some sleeping genes in the cancer cell of course the most probable explanation. Gordon has proved that the genome in the nucleus from a somatic cell may be awakened and activated in the right surroundings — in the cytoplasm of an egg cell. This means the totipotentiality of each cell nucleus. These findings may have been discussed.

In summary it must be said that this volume contains a wealth of information and an excellent bibliography of American contributions. Modern oncology has to work with these questions in the future. Even if many problems may seem to be basic the concept of paraneoplasia is clinical. It must therefore be regretted that some authors obviously have had little clinical experience. In spite of this the book may be strongly recommended to all workers both in the laboratory and at the bedside who are interested in fundamental biological problems connected with cancer. And who is not?

Jan G Waldenström

Pharmacology of gastrointestinal motility and secretion volume 1 and II Edited by Pamela Holton
665 pp £18.00 Pergamon Press Oxford, England 1973

The experimental study of gastrointestinal motility and secretion has made rapid progress during the last decade. To a large extent this development is due to advances in preoperative chemistry which has made isolation of several gastrointestinal peptide hormones possible. The rapid progress in the field has recently generated several reviews dealing with various aspects of gastrointestinal motility and secretion. The present survey is an attempt to give the whole panorama of current gastrointestinal physiology and pharmacology with some additional clinical information. It is published as a section of the *International Encyclopedia of Pharmacology and Therapeutics*. Pamela Holton, reader of physiology at St. Mary Hospital Medical School in London has been entrusted with the editorship. She has assembled 25 well-known scientists as authors of the 18 different chapters of the two volumes.

The book is opened with a discussion of the pharmacology of salivary secretion. Three chapters are dealing with the chemistry and biological actions of gastrin, cholecystokinin and secretin. Two separate chapters are presenting different opinions on the role of histamine in the regulation of gastric acid secretion. Other parts include papers on effects of catecholamines on gastric secretion and the role of adenylyl cyclase and carbonic anhydrase in the gastrointestinal tract. Several other topics are also presented, such as a discussion of drugs used in the treatment of peptic ulcer and drugs for disordered motility of the alimentary tract.

The second volume is closed with a chapter on endocrine hormones producing gastrointestinal symptoms.

The book does not include any general discussion of the pharmacology of the liver. The chapters mostly cover the pertinent literature up to mid-1971. Some chapters are supplemented with a short addendum covering the time until 1973. The greater part of the books is occupied by discussions of basic pharmacological and physiological mechanisms. Several of these papers are well written, and give thorough information about the present stand-point of science in respective field. However other chapters are not fully up-to-date which is partially due to the rapid pace of science making the reviewers and publishers to lag behind. The clinical topics are not given the same thorough consideration which has been paid to many of the more basic physiological phenomena. This is probably reflecting the fact that the recent advances of basic science have so far only influenced the clinical practice to a moderate degree. However there are some passages in the clinical text which are clearly out-of-date and does not reveal the significant progress which after all has been made for example in the area of diagnosis of hormone-producing tumours as a result of the introduction of specific radio-immunoassays.

On the whole the two volumes contain a lot of relevant and fresh information mainly along the field of basic physiology and pharmacology of the gastrointestinal tract. Therefore the book is of value as an encyclopedia for the experimental worker within the field. It should also be of interest for the clinician interested in new aspects of and speculation on the pathophysiology of malfunction of the digestive organs.

Lars Sjodin Stockholm

THE VAGARIES OF ERYTHROLEUKAEMIA

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Abstract: A 10-year material of erythroleukaemia from department of medicine subspecialized in haematology is presented. A total of 14 cases fulfilled the following criteria for erythroleukaemia: erythroblastic hyperplasia of the bone marrow, either normo- or megaloblastic ineffective erythropoiesis, no evidence of vitamin B₁₂ or folic acid deficiency, relatively few myeloblasts in the bone marrow in the initial stage, lethal outcome and leukaemic infiltrates in later bone marrow aspirates and often in the organs at autopsy. The great variability in the clinical picture, in the morphology of the red blood cell precursors and in the course of erythroleukaemia is emphasized, and the connection with other so-called myeloproliferative disorders is illustrated by the common transformation to acute myeloid leukaemia and the development of erythroleukaemia from polycythaemia vera and chronic myeloid leukaemia.

The disorders commonly grouped under the designation of erythroleukaemia, erythraemic myelosis or Di Guglielmo's syndrome are among the most intriguing in haematology. The term 'myeloproliferative syndrome' introduced by Dameshek (4) has been applied also to this group of diseases and it is generally agreed that one is dealing with neoplasia. The problems concerning nomenclature and classification of the Di Guglielmo syndrome have recently been reviewed (6, 20).

The aim of this paper is to present a 10-year period of experience with this group of disorders from haematologically subspecialized medical department commonly treating cases of acute leukaemia. We want especially to call attention to the difficulties in establishing the diagnosis early, the great variability of cases which may be categorized as erythroleukaemia, and the relationship between these diseases and other haematological conditions.

MATERIAL

The patients represent cases which by routine have been registered as erythroleukaemia in this department in 1965-1971. No attempts have been made to perform a prospective study by using predetermined definitions for entry into this series. The diagnosis has been established by the same group of haematologists as part of their routine work in haematology. Retrospectively it appears that the following criteria have been essential for the diagnosis of erythroleukaemia (Tables I and II).

(1) Erythroblastic hyperplasia of the bone marrow which was either normoblastic, more or less atypical megaloblastic, or just displayed abnormal cytological features. (2) Evidence of ineffective erythropoiesis from the discrepancy between bone marrow erythroid activity, the degree of anaemia, and the relative lack of reticulocytes. (3) No evidence of vitamin B₁₂ or folic acid deficiency. (4) Relatively few myeloblasts compared to erythroblasts in the bone marrow at the initial stage of the disease. (5) Short duration of the disease with lethal outcome. (6) Leukaemic infiltration in later bone marrow aspirates or in various organs at autopsy.

The mode of suspicion of leukaemia was high when the number of myeloblasts exceeded 5%. Irrespective of the myeloblast percentage, leukaemia was thought to be proven when Auer bodies were present in the myeloblasts. It is of interest to note that in some cases Auer bodies were only found upon retrospective bone marrow examination (see below).

The total number of patients fulfilling these criteria for erythroleukaemia in the 10-year period was 14. During the same time 112 patients were treated for acute myeloblastic leukaemia, 5 for monocytic and 49 for acute lymphoblastic leukaemia. It should be noted that, with two exceptions, only patients above the age of 15 years were admitted to the Haematology Division during this period.

METHODS

All initial bone marrow samples were reexamined by one of us (S.K.). The number of cells counted was 100-200. In addition, the slides were carefully screened for

Table I Laboratory results (blood) in 14 cases of erythroleukaemia at first admission

Case no.	Age (y)	Sex	Hb (g/100 ml)	Reticulo-cytes (%)	MCV (μ^3)	Leuko-cytes/ μ l	Thrombo-cytes/ μ l	Mys. lo-blast (%)	Erythro-blast (%)	Serum iron (μ g/100 ml)
1	17	♀	7.0	8	115	6 800	119 000	78	0	166
	75	♀	3.6	77	110	1 200	1 000	0	0	39
3	62	♀	5.5	5	106	18 200	20 000	25	1	229
4	61	♂	9.5	8	91	400	34 000	0	8	87
5	35	♂	6.6	1		6 700	68 000	2	2	
6	41	♂	5.5	1	100	700	84 000	0	79	260
7	74	♂	5.0	3	101	400	1 000	8	0	
8	60	♀	7.5	12	123	600	189 000	0	0	159
9	14	♂	6.4	4	115	2 300	76 000	10	10	21
10	70	♀	6.1	6	93	600	4 400	0	2	168
11	28	♀	6.5	18	111	26 900	84 000	22	0	250
1	39	♀	8	47	101	6 000	80 000	8	18	154
13	49	♂	3.6	4	91	400	32 000	0	0	314
14	81	♂	6.3	12	97	570	11 000	0	0	155
Normal range										90-150

Examined in another hospital just before transfer

Table II Bone marrow results in 14 cases of erythroleukaemia (first sample after onset of symptoms)

Case no.	Cellularity of bone marrow smear*	Megalo-blast (%)	Abnormal eryth-roblasts† (%)	Normo-blast (%)	Total eryth-roblasts (%)	Remark
1	++	0	51	19	70	Rather typical megaloblastic RBCP + Auer bodies present
3	+	7	45	0	55	Typical megaloblastic RBCP
4	++	5	18	26.5	44.5	Moderately megaloblastic RBCP
5	+	3	9	73	82	Atypical myelocytes
6	+	2	1	36	37	Atypical megaloblastic RBCP
7	-	1	6	60	66	Atypical megaloblastic RBCP quite bizarre; myeloid precursors somewhat atypical
8	(+)	1	1	40	51	Normoblast slightly atypical. Auer bodies seen in neutrophil precursors
9		2	0	64	64	Abnormal RBCP were megaloblastic but rare normoblastic cells appeared normal
10	+	10	45	11	66	No atypical RBCP seen
11	+	9	31	45	76	Only slight megaloblastic changes. Differentiating neutrophils atypical
12	+	7	7	66	73	Abnormal RBCP more odd than megaloblastic. Scattered binucleated RBCP
13	+	3	5	89	94	Only slight megaloblastic changes. Auer bodies present
14	+	1	0	76	76	Megaloblastic changes very slight
15	++	9	11.5	30.5	47	N. typical RBCP seen. Very small my. locytes (pseudo-Pelger cells)
16	++	9	11.5	30.5	47	Abnormal RBCP more odd than megaloblastic. Several binucleated RBCP

++=highly cellular + = normocellular (+) = hypocellular slide
Including more or less typical megaloblasts*

Serum transferrin ($\mu\text{g}/100\text{ ml}$)	Serum B_{12} ($\mu\text{g/l}$)	Serum folic acid ($\mu\text{g/l}$)	Serum bilirubin ($\text{mg}/100\text{ ml}$)
241	170		0.6
14	116	7	0
266	128	7	1.0
	171	4.5	1.4
			1.8
17	1090	7	0.9
	Normal	Normal*	1
305	30	11	0.4
16			
146	360	10	0.8
390	900		0.4
	430	6	1.4
Normal*	Normal*	1	0.7
41	607	6	0.9
30-480	150-800	4-18	<1.0

special features such as Auer bodies and other signs of dysdifferentiation in the neutrophilic line such as pseudo-Pelger cells. Chromosome studies were performed on bone marrow samples from 7 of the patients according to the method of Tjio and Whang (23).

RESULTS

Table I presents age and sex distribution of the patients as well as laboratory findings at the first admission to the department.

Table II gives the result of reexamination of the first bone marrow sample. The Table contains in-

formation about the cell lantcy of bone marrow smears, the number of myeloblasts, the relative frequencies of normal and abnormal erythroblasts with specification of the type of abnormality and special findings such as Auer bodies and dysdifferentiation in the myeloid series. The abnormalities in the erythroblastic cells comprised both typical and atypical megaloblastic changes, abnormal red blood cell precursors (RBCP) without megaloblastic changes have been designated as 'odd'.

Table III shows the findings in chromosome studies in seven of the patients.

Fig. 1 depicts the course of the disease in all 14 cases, starting from the onset of symptoms and indicating the time of the first bone marrow sample and the time when the diagnosis of leukaemia was suspected and/or established. Fig. 2 shows the survival curve in the 14 cases, calculated from the time of the first bone marrow sample.

CASE REPORTS

Case 1

A 17-year-old woman in 1964, after symptoms of anaemia for 3 months, she was admitted to local hospital where severe macrocytic anaemia and subnormal levels of thrombocytes and leucocytes without immature cells were discovered. The bone marrow was hyperplastic with atypical megaloblasts. Serum B_{12} normal. She was given blood transfusions and iron, but the symptoms reappeared and 6 months later she was admitted to this

Table III Results of chromosome studies in 7 cases of erythroid leukaemia

Case no.	Time after admission	No. of chromosomes	Site of karyotype anomaly	Other remarks
2*	2 day 6 week 11 week	42	D, E & F	Ring chromosome present
3	1 day 8 week 10 week	Normal		
5	1 week	44 (80% of metaphases)	karyotyping not possible	Marker chromosome (80% of metaphases) viz. small metacentric chromosome half the size of group C chromosomes
6	days 74 week	Normal Normal		
11	1 week	Normal		
12	1 week	48	E & G	All metaphases containing Philadelphia-chromosomes
14	1 week	54	karyotyping not possible	Small acentric fragment

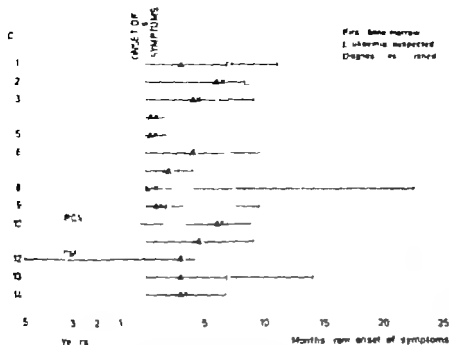


Fig. 1 Course of the disease in 18 cases of erythro-leukaemia from the onset of symptoms. Duration of preceding PCV and CVL indicated on the left.

department. There was no hepatosplenomegaly and no enlarged lymph nodes. From the blood and bone marrow findings the diagnosis of erythro-leukaemia was established. After treatment with 6-mercaptopurine the myeloblasts disappeared from blood and marrow, whereas anaemia and megaloblastic changes remained. An attempt to circumvent possible enzymatic block treatment with thymidine (total dose 14 g/3 days) was without effect on the megaloblastic marrow. After treatment with prednisone (40 mg/day) and transfusions, the patient was discharged but died 1 month later in febrile anaemic state at the local hospital by autopsy.

Case 3

A 74-year-old woman. At the age of 40 spondylitis had been treated by several series of radiotherapy (dose unknown). In May 1965 she was admitted to the department after having suffered from increasing fatigue for 6 months. There was no enlargement of the liver, spleen or lymph nodes. The tongue was devoid of papillae and serum B_{12} moderately reduced (116 $\mu\text{g/l}$) but the Schilling test I was normal. The marrow was completely dominated by typical megaloblasts. During treatment with massive B_{12} some restoration of the papillae of the tongue and a marked reticulocytosis occurred but the RBC remained unchanged. The marrow was still dominated by erythroid precursors, which now were much less megaloblastic. From the finding of number of myeloblasts and a stem line with 42 chromosomes, erythro-leukaemia was suspected. Therapy with folic acid had no haematological effect, and the patient received several transfusions before discharge. She was readmitted about 1 month later with thrombocytopenia and gastrointestinal bleeding. Now the white cells in the blood and bone marrow were dominated by myeloblasts,

and there was a conspicuous absence of erythropoietic cells. Death occurred in early August 1965. At autopsy the bone marrow, liver, spleen and lymph nodes were infiltrated with immature mononuclear cells.

Case 4

A 61-year-old woman. Ten years earlier the patient had been radically operated upon for carcinoma of the uterus. Radiotherapy (400 R) was given 3 years before admission because of recurrent uterine abscesses. She was admitted to the department in June 1965 after having suffered from symptoms of anaemia for about 9 months. There was no hepatosplenomegaly or enlargement of the lymph nodes. Erythro-leukaemia was suspected but the patient was only treated with transfusions. Three weeks later stenosis of the trachea with increasing stridor demanded tracheostomy. Tracheal biopsy showed infiltration with immature mononuclear cells. The blood and bone marrow were now dominated by myeloblasts. Despite treatment with 6-mercaptopurine the condition rapidly deteriorated and death occurred a few weeks later. At autopsy the bone marrow, liver, spleen, kidneys, tonsils and mucosa of the trachea were infiltrated by immature mononuclear cells.

Case 5

A 61-year-old man. In April 1965 he was admitted to another department because of anaemia, discovered by routine examination. From blood and bone marrow examinations erythro-leukaemia as well as myelofibrosis was considered. He underwent exploratory laparotomy because stenosis of the ilium was suspected. This was not confirmed. When he was seen in this department in August 1965 there was no hepatosplenomegaly and no

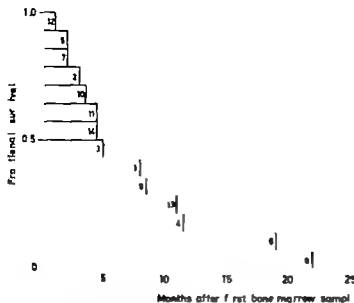


Fig. 2 Survival in 13 cases of erythroleukemia from the time of the first bone marrow sample. The numbers refer to the case reports and the halting indicates cases treated with cytotoxic agent.

enlargement of lymph nodes. As he gradually developed pancytopenia and hypocellularity of the bone marrow treatment with prednisone (40 mg/day) was instituted. Later on, when leucocytosis as documented by ^{51}Cr labelling (blood $T_{1/2}$ 10.5 days splenic accumulation) splenectomy was made. Shortly after the blood showed an increasing shift to the left in the myeloid series and he died 1 month after the operation. Autopsy revealed hyperplastic bone marrow with dominance of erythroblasts, many of which showed nucleocytariness. Small leukaemic infiltrates were seen in the liver lymph nodes and adrenal glands.

Case 5

A 35-year-old man, who was admitted for pancytopenia to this department in Feb. 1966. For 2 weeks he had suffered from fever and pains in the chest. There were enlarged lymph nodes in the neck and in the axillae but the liver and spleen were not enlarged. Bone marrow examination and chromosome studies aroused suspicion of acute leukaemia. Therapy with 6-mercaptopurine was instituted but his condition rapidly deteriorated with pancytopenia. A new bone marrow sample was dominated by erythroblasts, most of which were megakaryoblasts. Many atypical myeloblasts were also present, confirming the diagnosis of acute leukaemia. Death occurred in April 1966. No autopsy.

Case 6

A 41-year-old man. In June 1969 he received transfusions in another hospital after having suffered from anaemia for 3 months. On admission to this department 1 month later physical examination was normal. The bone marrow material was very scanty but sufficient for the diagnosis of erythroleukaemia. Auer bodies were present in the myeloblasts. The patient was treated with vincristine and methotrexate without any effect. During the

following 17 months he was readmitted 13 times for transfusions. He had no fever or haemorrhagic diathesis. The bone marrow was still hypoplastic and consequently cytotoxic treatment was not reinstituted. From August 1970 there was progressive pancytopenia and increasing number of myeloblasts in the blood but he was relatively well until Feb. 1971 when he suddenly developed subdural haematoma. The haematoma was evacuated, but the patient died a few days later. At autopsy the bone marrow was severely aplastic. There was moderate splenomegaly but no mature myeloid cells were seen in the organs.

Case 7

A 74-year-old man with symptom of rheumatic heart disease for 4-5 years. For about 1 month he had suffered from anaemia when admitted to local hospital in 1967. A severe pancytopenia was discovered and treatment with prednisone (30-60 mg/day) was instituted. For short period he received treatment with stanozolol and folic acid. The condition remained unchanged and when bone marrow examination showed erythroleukaemia, he was transferred to this department. There was no lymphadenopathy or enlargement of liver and spleen. A new bone marrow examination confirmed the diagnosis by showing hypocellularity with predominance of bizarre megakaryoblastic erythropoiesis as well as significant number of abnormal myeloblasts and promyelocytes. Treatment with methotrexate and vincristine was attempted without result and 1 month later he died from pneumonia and septicæmia. Autopsy showed lobar pneumonia and hypoplastic bone marrow. No leukaemic infiltrations were found in the organs.

Case 8

A 60-year-old woman. In 1968 she was admitted to the Department of Dermatology because of chronic eczema.

were present. In addition the serum concentration of vitamin B₁₂ was reduced and the patient's tongue was devoid of papillae. There was some doubt concerning the diagnosis but a trial with vitamin B₁₂ was made. This resulted in normalization of the epithelium of the tongue and a reticulocytosis which however was not followed by a rise in the Hb concentration. Soon the picture of the bone marrow changed to a myeloblastic pattern. A ring chromosome which was present all ready when the patient was suspected to have pernicious anaemia remained throughout the course of the disease even at the time when the bone marrow was completely dominated by myeloblasts.

The fourth interesting case is patient 8. There was an early suspicion of erythroleukaemia but no definite diagnosis could be made. The alternative diagnosis was haemolytic anaemia and prednisone was given resulting in a very definite improvement of the general condition and rise in the Hb concentration. However some months later it became apparent from blood and bone marrow cytology that she had AML. With mild antileukaemic chemotherapy and transfusions the patient was kept alive for 2 years after the first bone marrow examination. It should be noted in this context that prednisone is not or only occasionally effective in AML, of which we consider erythroleukaemia to be a variant.

General features

The term erythroleukaemia was first coined by Dameshek in 1917 (10) who later extended the concept to other conditions which we now know are not neoplastic (11). Dameshek (4, 5) later tried to make a summation of the various neoplastic diseases of the bone marrow such as the acute leukaemias including erythroleukaemia, CML, PCV, myelofibrosis etc. under the general heading "myeloproliferative disorders". There is no doubt that many of the neoplastic haematological disorders are intimately connected. Without going into a detailed discussion of this vast problem we should like to point to the fact that several patients in this series confirm the intimate linkage between the various neoplastic haematological disorders. Thus patient 10 had PCV terminating in erythroleukaemia. This course has been described previously (2, 7, 21). The transition from CML to erythroleukaemia (patient 1) has also been reported before (15, 19).

The frequency and types of chromosome abnormalities found in our study are in accordance with earlier reports (3, 14). In case 2 it was demonstrated that the same marker chromosome was present at the time when the bone marrow was completely dominated by erythroblasts and at the time when the marrow was predominantly myeloblastic. Regarding case 17 it was noted during the erythroblastic phase that all metaphases contained two Ph chromosomes.

Although it is very much used the term "myeloproliferative disorder" seems less appealing to us. The point is that in many of these disorders the marrow cells are not growing faster but rather more slowly than in the normal state (13). This is also the case in erythroleukaemia (1). Therefore we would rather use as a general denominator for these conditions the term "*neoplastic disorder of the common haemopoietic stem cell*". Dependent on so far unknown circumstances the defect may preferentially lead to involvement of one of the cell lines or the picture may be that of a more generalized bone marrow disorder. The limits are not sharp and during the course of the disease changes from one type to another may easily occur. The diagnosis may in many instances depend on the stage of the stem cell disorder in which the patient is seen by the physician.

It has been shown that CML arises from a change in the common haemopoietic stem cell (24) and there is also good evidence that AML including erythroleukaemia arises in this cell (16, 17). This may be true also for myelofibrosis (18). As regards PCV no definite evidence exists but maybe the more refined methods of chromosome studies by the banding technique will shed light on these problems.

In some respects erythroleukaemia shows differences from other acute leukaemias but perhaps this is merely an expression of how the influence of external factors upon human leukaemias can be studied in vivo. Thus control mechanisms of erythroid proliferation in erythroleukaemia are not completely lost as determined from the marrow response to blood transfusions (1, 8) also the pattern of marrow cell growth in vitro resembles more closely that of a preleukaemic state (9).

Fig. 2 shows the fate of the patients after the first bone marrow sample. It is seen that the survival follows the usual survival of leukaemias i.e. an exponential function. It is also apparent that it

made no difference whether or not chemotherapy was given. It must be noted in this context however that the chemotherapy administered was deliberately not erythrosuppressive in view of the generally accepted fact that erythroleukaemias are particularly resistant to chemotherapy. In addition it must not be overlooked that the series runs from 1961 to 1971: in the early part of this period some of the now very efficient drugs were not yet available. At the present time, with a larger choice of drugs, a more active therapeutic attitude towards erythroleukaemia may be preferred.

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APLASTIC ANAEMIA, CONSIDERATIONS ON THE PATHOGENESIS

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Abstract Modern concepts of haematopoiesis and its regulation are reviewed. Observations from several forms of aplastic anaemia are analysed in the light of this new knowledge. The pathogenetic mechanisms in the aplasia caused by chloramphenicol is probably stem cell damage of an autoimmune or idiosyncratic nature. Chlorpromazine aplasia is apparently due to constitutionally defective DNA synthesis so that normal compensatory reaction cannot take place against the inhibiting effects of the drug on DNA synthesis. The chlorpromazine aplasia is normally transient, since transit cells of the bone marrow—and not the stem cells—are affected. The hepatitis-associated aplasia may possibly be precipitated by an autoimmune attack on multipotent stem cells or on transit cells of one or more cell lines after virus-induced change of antigenicity of the cell surface. The thymoma-associated aplasia may likewise be due to autoimmunity directed against transit cells. Finally there are indications that one Fanconi aplasia may be pathogenetically heterogeneous, with constitutionally defective stem cells, transit cells, or stromal cells. Otherwise stromal failure rarely seems to play a role in human aplastic anaemia. Furthermore, stem cell exhaustion probably does not exist. Neither stem cell exhaustion nor situations with missing or defective haematopoiesis apparently produce aplasia on their own. A research strategy supplementary to the routine haematological examinations is proposed. It is recommended that modern immunological techniques in particular should be applied in combination with stem cell assays.

Aplastic anaemia is characterized by hypocellular or well-lar bone marrow and by haematopoietics which is grossly insufficient in bone marrow as well as in extramedullary sites. This anaemia often runs a chronic course and the majority of the patients die from infection or bleeding.

The most important causes of aplastic anaemia are as follows:

- Acquired: 1) Physical agents (ionizing radiation)
- 2) Chemical agents (a) dose-dependent e.g. benzene, cytotoxic drugs (b) idiosyncratic, viz. dependent on individual susceptibility e.g. chloram-

phenicol, antiepileptic drugs, chlorpromazine, 3) Associated with virus hepatitis 4) Associated with thymoma 5) Idiopathic. Constitutional Fanconi anaemia with infants.

Almost half the cases are idiopathic and the majority of the remaining ones are caused by drugs.

In most cases very little is known about the pathogenesis of the disease. This is largely due to lack of convenient animal models. In the recent years new relevant methods have been developed, however, and the possibilities for thorough study of probable pathogenetic mechanisms has improved considerably.

RELEVANT EXPERIMENTAL FINDINGS

Results obtained by experimental haematologists during the last decade may throw some light on the pathogenesis of aplastic anaemia. The attention has more and more been focused on the stem cells—their properties and growth regulation.

It is now firmly established from experiments with rodents that multipotent haematopoietic stem cells do exist (Fig. 1) (37). The distinguishing feature of stem cells is their ability to give rise both to differentiating daughter cells and to new stem cells. The differentiating and maturing cells will on the other hand lose—probably gradually—the capacity for self-renewal. These members of the transit populations are destined to die when their period as functional end cells is over. Some of the mature lymphocytes are apparently an exception from this rule, since they may renew themselves during an immune reaction. Furthermore, some lymphocytes are potentially very long-lived and this explains why they are the only remaining blood cells in significant numbers in chronic aplastic anaemia.

Normal haematopoiesis during steady state conditions can—at least in the mouse (4) and in the rat (20)—take place even though most of the stem cells do not proliferate. It is sufficient that a minority of them, perhaps 15% are in generative cell cycle, dividing or preparing for division. The existence of large reservoirs of non-dividing stem cells might explain why

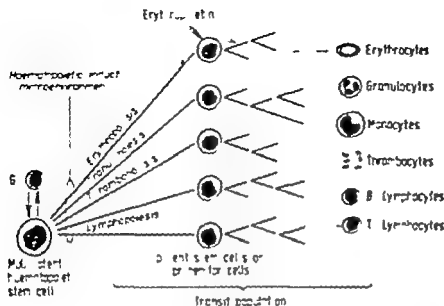


Fig. 1 Haemac cell formation from multipotent stem cell. The hierarchy of multipotent and unipotent stem

or progenitor cells and the established regulating factors.

some aplasia condition do not manifest themselves until week or month after the action of toxic agent, namely when dormant stem cell has been activated in order to maintain normal haematopoiesis.

There are large gaps in our knowledge about the regulation of haematopoiesis. *Erythropoiesis* permits the unipotent erythroid progenitor cells to mature further to the stages of morphologically recognizable erythroblasts. It probably also increases the rate of proliferation of these progenitor cells and their immediate descendants (19, 39, 46). There is good evidence

the existence of an analogous thrombopoiesis (37), the existence of specific granulopoiesis (17), but been conclusively proved. However it has not been possible to associate any of the clinically important aplasia, anaemia, or defects protein metabolism.

Very suggestive evidence indicates that the micro-environment of the stem cells may influence both their direction of differentiation and their proliferation rate. For example transplanted stem cell settling in the spleen of an irradiated mouse will give rise to more erythropoiesis than granulopoiesis, whereas the opposite situation is found in the bone marrow (18).

Experiments with two types of hereditary anaemia in the mouse (i.e. the W/W^a and the Sl/S^h) anaemias, have given information on possible pathogenic factors in aplastic anaemia. If normal adult-type (1) bone marrow cells are injected into lethally irradiated normal or unirradiated W anaemic mice some of the stem cells will settle in the spleen, start proliferating and give rise to large clones of blood cells. After one week some of the clones located near the splenic capsule will be large enough to form visible nodules. Deeply located and smaller nodules can be examined by microscopy. This is the now classical spleen

colony assay for stem cells (54). The results obtained when different combinations of 1 W/W^a and Sl/S^h mice were used in this way are given in Table I. The most likely interpretations of these experiments are that in the W mouse there is a genetic defect that prevents the rapid formation of large clones of own cells, whereas the Steel mice have normal stem cells. Here a hereditary defect in the stroma disturbs the cell growth. These interpretations have received additional support from other types of experiment (48). The importance of the stroma has been demonstrated in experiments where spleen containing stem cells are transplanted subcutaneously to the peritoneum, or under the renal capsule (Table II). The finding that a W spleen graft can improve the anaemia of Steel mouse indicates that blood-borne normal stem cells of the recipient can find fertile soil for haematopoiesis in the normal stroma of the graft. Several investigators have confirmed that haematopoietic progenitor cells in blood-borne in both man and mice (3, 16, 30, 34).

These experiments with anaemic mice therefore point to two potentially important mechanisms in bone mar-

Table I. Formation of haematopoietic spleen colonies in bone marrow cell from normal or anaemic mice injected into irradiated normal or anaemic recipient.

Donor	Recipient		
	1	W/W ^a	Sl/S ^h
1	Microcolonies	Microcolonies	-
W/W ^a	Microcolonies	Microcolonies	-
Sl/S ^h	Microcolonies	Microcolonies	-

Table II Effect on haematocrit of spleen transplants from normal and aemic or between different kinds of aemic mice

Donor	Recipient	Improved (%)
1	SUSM	13/15
1	W/W ^a	1/6
SUSM	W/W ^a	8/9
W/W ^a	SUSM	8/10

Normal splenic stroma transplanted to SUSM mice may be colonized by circulating, normal, host stem cells. Whereas normal stem cells present in the grafts given to W/W^a mice may colonize the normal haematopoietic stroma of the hosts. Modified from Bernstein (8)

row aplasia, i.e. too few or defective stem cells on the one hand, and defective haematopoietic stroma on the other.

A *maturation arrest* of the bone marrow cells has been claimed—and widely accepted—to be an important pathogenetic factor (13). There is, however, no sound experimental evidence for aplastic anaemia being the result of a deficiency of maturation factors or the presence of maturation inhibitors (11). On the other hand, alternative explanations can be offered to explain the myelogram in such cases.

The first is that different cell types carry specific cell surface antigens (24). Within the haematopoietic system there exist separate and specific surface antigens, for example on granulocytes, T lymphocytes, B lymphocytes and macrophages (44, 45, 51, 56). The concentration of these surface antigens may increase with increasing maturation of the cells, as shown for erythrocytes in Table III. Nevertheless it is possible that stem cells, too, possess at least one almost prime antigen (25). The implications would then be that humoral or cell-mediated autoimmune attack could selectively eradicate mature and semimature cells of one or more cell lines. In other cases the stem cell or stromal cells might be preferentially damaged.

An alternative explanation of maturation arrest could be that the myelogram represents "snapshot of re-

generative haematopoietic process that has just reached for example the metastylocyte stage (49).

In some types of aplastic anaemia, e.g. in benzene poisoning the aplastic stage is reached after phase 4 bone marrow hyperplasia. This progression of the disease might suggest that long-lasting and stimulus to proliferation and differentiation could have a *thymic stem cell pool* with subsequent aplasia as result. This suggestion gains support from experiments with serial bone marrow transplantation between lethally irradiated syngeneic mice. After 3-4 bone marrow transfers the protective capacity of the marrow apparently lost (7). Although the stem cells of such transplants seem to grow poorly in bone marrow, the growth rate in the spleen is apparently normal. If they are let alone for some week in the bone marrow of the irradiated hosts, they will recover normal proliferative capacity (57). Finally, the total stem cell population of old mice is not reduced (7). These findings indicate that stem cell exhaustion will become important when an additional pathogenetic factor is not such radiation damage of stromal (1).

The pathogenetic mechanisms are fairly well elucidated in the aplastic condition caused by *radiation and chemicals* that damage haematopoietic stem cells in dose-dependent manner (14, 15). It is possible that stromal damage may be important in the acute radiation syndrome since it is known from animal experiments that rather modest radiation dose will lead to rupture of bone marrow sinusoids and haemorrhage into the parenchyma (1). Furthermore, secondary aplastic phase due to fibrosis and vascular damage produced in rat by large doses of X-ray irradiation experiments by Knoke and Crosby (77). They have suggested that many cases of hitherto one-planned bone marrow failure may depend on stromal damage when aplastic region persists in marrow cavities that are otherwise normal or hyperplastic. One of their experimental models aplasia persists after supralethal irradiation and transplantation of allogeneic, but not syngeneic marrow. This was interpreted as indicating synergistic effect between radiation and graft error, host reaction against stromal components. However, the possibility was not excluded that stem cell proliferation is suppressed for other reasons (19, 35, 5) and not because the microcirculation is damaged. Therefore the only experimentally verified condition of bone marrow failure caused by stromal insufficiency is the above mentioned high-dosage irradiation aplasia (40).

CLASSIFICATION SCHEME FOR APLASTIC ANAEMIA

A classification of possible pathogenetic factors in aplastic anaemias listed in the introduction can now be attempted. 1. Ionizing radiation. 2. Chemical cytotoxicity (a) direct, dose-dependent; (b) conditional (idiosyncratic); (c) immunological. 3. Viral cytotoxicity (a) direct; (b) immunological. 4. Autoimmune cytotoxicity. 5. Cell exhaustion. 6. Hereditary defect. It should be noted that any pathogenetic factor that causes chronic aplasia by attacking the transit populations of

Table III. Antigen density on various cell types from foetal liver as determined by electron microscopy after application of ferritin-labelled anti-erythrocyte antibody

	Antigenic sites/ μm^2
Liver epithelial cells	70
Proerythroblasts	90
Polychromatophil erythroblasts	40
Erythrocytes	500

Adapted from Kisalo et al. (38)

bone marrow cells must act continuously. A single damaging event of limited duration and great intensity may be sufficient when the attack is directed against stem cells or stroma.

The classification of the clinically most interesting types of aplastic anaemia into this scheme cannot yet be carried out with an adequate degree of confidence. Clinical observations give some clues, however, so that some qualified guesses can be made.

SEMI-SPECULATIVE CLASSIFICATION OF CLINICALLY IMPORTANT APLASTIC ANAEMIAS

The aplastic anaemia caused by chloramphenicol will most often run a chronic and fatal course, but recovery has been reported (41). In a few cases it has been cured by transfusion of haematopoietic cells (Hjort and Björum, personal communication). This indicates that the stem cells are the target cells of the pathological process. The few remaining granulocytic progenitors seem capable of dividing and differentiating normally in culture (41) and the ³H-thymidine labelling index of erythroid blasts *in situ* is apparently within the normal range (40). These findings are additional evidence for a stem cell disease.

The aplasia may often arise even after a small total dose of chloramphenicol or late after finishing the course of drug treatment (60). A dose-dependent and reversible pancytopenia may also be seen after chloramphenicol, probably because the drug inhibits the attachment of mRNA to membrane-bound ribosomes, leading to a defect in protein synthesis in mitochondria (4, 60). It is unlikely that the same mechanism works in the dangerous and irreversible form of bone marrow depression (60) since there is no evidence for abnormal accumulation of chloramphenicol in such patients. The late occurrence after stopping the drug also argues against this possibility.

The two most probable mechanisms of damage therefore seem to be autoimmunity and drug idiosyncrasy. A model for the latter mechanism found among the haemolytic anaemias (9). It is conceivable that a hereditary defect in stem cells makes them susceptible to damage by certain chemical compounds. However, one would expect that an enzyme defect that predisposes to chloramphenicol damage of stem cells should also be present in the more mature cells. These cells would then also be susceptible to damage, but it

seems that they are generally unaffected (9). It has been reported, however, that rather high concentrations of chloramphenicol added to bone marrow cultures from formerly aplastic patients may depress DNA synthesis to a slight extent. A similar depression was found for bone marrow cells from two fathers, but not from the mothers of aplastic patients. Even higher doses were needed to affect normal control marrow (60). Since the doses were quite high (25–40 µg/ml) and since the majority of the stem cells probably do not engage in DNA synthesis during the period of drug exposure, these studies do not give an unequivocal answer to the question of the pathogenesis of the aplasia.

Except for the reports of two pairs of identical twins suffering from chloramphenicol aplasia, there is no evidence for a genetic predisposition (9, 40, 43). Even if there was a genetic element, this would not solve the dilemma, because the immune response to specific antigens is clearly influenced by genetic factors, as shown by the existence of responder and non-responder strains among rodents.

On the other hand, the occurrence of a relative bone marrow lymphocytosis and plasmacytosis (40) and patchy distribution of the aplasia (60), maturation arrest (9), involvement of one cell line only (43) and during the early stages haematopoietic cell hyperplasia (9, 60) is perhaps best compatible with an autoimmune mechanism. There are indications that autoimmunity is important in at least some types of aplastic anaemia, since some patients have improved after unsuccessful bone marrow transplantation. The feeling is that this might have been due to the immunosuppressive conditioning of these patients (5).

This autoimmune mechanism might be similar to one of those proposed for methylidopa-treated patients with haemolytic anaemia (59). Chloramphenicol or one of its metabolites could act as antigen after being bound to a soluble macromolecule or as a stem cell antigen. The humoral or cell-bound antibodies would then be directed against chloramphenicol (metabolite) or against the altered stem cell antigen, and they might in both cases cross-react with normal stem cell antigen. Alternatively, chloramphenicol (metabolite) might induce immunocompetent cells to commit the 'horror autotoxicus'—reacting against normal stem cells.

The autoimmune reaction that can be triggered by methyldopa (and possibly also by chloramphenicol) may be short-lived after discontinuation of the drug. Therefore the existence of the above mentioned hypothetical mechanisms is not contradicted by the few successful bone marrow transplantations performed in chloramphenicol aplasia.

The bone marrow aplasia caused by *chlorpromazine* is fairly common among patients in mental hospitals (42). Blood examinations disclose agranulocytosis and in some cases thrombocytopenia. The bone marrow depression is dose-dependent. The condition is easily reversible so that anaemia rarely develops. These findings strongly suggest that chlorpromazine affects the transit populations of the bone marrow. After normalization the patients can take moderate doses of the drug without serious relapse. This argues against immune mechanisms and rather indicates an idiosyncratic reaction. It has been shown that bone marrow from such patients has a subnormal uptake of ³H-thymidine *in vitro* even in the absence of the drug, suggesting a constitutional error in bone marrow cells. On the other hand chlorpromazine normally inhibits growth in several types of tissues. It therefore appears that individuals with constitutionally defective transit populations in the bone marrow will not be able to compensate for the growth-depressing effect of chlorpromazine (47).

The aplastic anaemia connected with *virus hepatitis* often occurs when the hepatitis is regressing or has been cured and the attack of hepatitis has often been a mild one (1, 10, 21, 22). Successful bone marrow transplantation (7, 57) is evidence against stromal damage and suggests stem cell disease. Since only one cell line may be hypoplastic (1, 22), the main attack may in some patients be directed solely against transit population. It has been suggested that the bone marrow is damaged by normal doses of substances metabolized in the liver but accumulation of such substances is unlikely since the liver may not be detectably damaged when the aplasia is discovered (10, 22). Auto-antibodies to thrombo- erythro- and leukocytes have been demonstrated (6) and the effect of glucocorticoid treatment has sometimes been satisfactory (10). Furthermore several types of virus can infect haematopoietic cells. Abnormal karyotypes, which could have been caused by virus

infection have been found in hepatitis aplasia (1). A reasonable hypothesis is therefore that hepatitis virus infects haematopoietic stem or progenitor cells so that new antigens e.g. coded by the virus, appear in the cell membrane making these cells immunogenic.

About 5% of patients with a *thymoma* have a haematological disease (18). This may be an aplastic anaemia, but more often it is an isolated erythroid hypoplasia. Accordingly the pathogenetic process may be directed against a transit population. The disease is prevalent in older people, and there is a connection with diseases considered to be of autoimmune nature (18, 47). The occurrence of antinuclear antibodies (28, 47) and a positive direct Coombs reaction (47) further support the existence of an autoimmune mechanism. Some patients improve when the tumour is removed (47). This indicates that the bone marrow condition was influenced by the thymoma. Since the tumours can belong to several histological types (18) it is probable that they cause the bone marrow aplasia indirectly by their effect on the remaining thymus tissue.

The *Fanconi anaemia* is probably a recessive hereditary disease (23, 41). It often manifests itself about the age of 8 and a prominent feature is the great variability of both the haematological findings and the type and degree of associated malformations (23, 26, 41). A large number of chromosome defects can be found in PHA-stimulated lymphocyte cultures (51) and sometimes in fibroblast cultures (26, 51). There appears to be an increased tendency to so-called malignant transformation by SV40 virus in fibroblast cultures from Fanconi patients (26). Bone marrow mitoses may or may not be abnormal in it (26, 51). Both megaloblastoid features and haemolytic anaemia have been reported (26, 41). It has been suggested that a tendency to chromosomal breakage is the hereditary defect (23). A cytopathic lesion could possibly occur either in stem cells, one or more transit cell types or in stromal cells.

PROPOSAL FOR A RESEARCH STRATEGY IN CASES OF APLASTIC ANAEMIA

Hypothetical pathogenetic mechanisms for several types of aplastic anaemias have been given above. Some of the mechanisms proposed are speculative and it is therefore important

rational strategy designed to unravel the secrets of these disorders. Accordingly some procedures supplementary to routine haematological investigations will be proposed. Multiple bone marrow biopsies could lead to the detection of patchy haematopoiesis and to a more meaningful quantification of lymphocytic and plasma cell infiltrations. A greater number of anaemias caused by hepatitis virus or by thymoma might be found if hepatitis and thymoma are specifically sought for with X-ray examination of mediastinum, virological examination and liver function test. Cytogenetic analysis may be mandatory in order to diagnose cases of Fanconi anaemia not accompanied by other malformations (6). If possible immunological studies should probably be done in most cases. These include one or more of the following procedures: humoral and cell-mediated responses to haematopoietic stem and transit cells in the presence and absence of the suspected offending agents; antinuclear factors; antierythropoietin antibodies.

Sometimes it may be worthwhile to investigate the cell kinetics of the transit populations, so that e.g. so-called maturation arrest could be more accurately described. Relevant studies might include H-thymidine labelling in *in vivo* cell life span determinations and measurement of ^{59}Fe kinetics *in vivo*. Finally it is important to know the quantity and proliferative rate of haematopoietic stem cells and cell line progenitor cells. There exist at present two different ways of procedures for such cells which can be applied to human material, i.e. the diffusion chamber culture technique (7-14) and the agar cloning technique (15).

New variant of aplastic anaemia will certainly appear in the future since new drugs are introduced at a rapid rate. This threat and the fact that most of the acknowledged types of aplastic anaemia are insufficiently elucidated underlines the necessity for establishing techniques which may disclose the relevant pathogenetic mechanisms.

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RED CELL 3-DIPHOSPHOGLYCERATE IN PATIENTS WITH HYPERTHYROIDISM BEFORE AND AFTER TREATMENT

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Abstract. Red blood cell 3-DPG has been found to be decreased in a group of 28 hyperthyroid patients. From careful analysis of the results taking into account Hb level the increase seems to be non-significant. According to the results of the present work red cell 3-DPG cannot be used as a parameter to evaluate the result of treatment in hyperthyroidism, as advocated by previous investigators.

Over 40 years ago Barow and Groschurth (1) were the first to observe that the oxyhemoglobin curve of hyperthyroid patients is displaced to the right. Lately this observation was confirmed by Gahlenbeck and Bartels (8), who also obtained the same results when triiodothyronine was administered to healthy persons. Gahlenbeck et al (9) found through experiments *in vivo* and *in vitro* that rat blood exposed to triiodothyronine showed decreased oxygen affinity. In 1967 two groups of researchers Charuiton and Lurie (4) and Benesch and Benesch (3), found independently of each other that an increase of red cell organic phosphates results in displacement of the oxyhemoglobin dissociation curve to the right thus improving the capability of the red cell to deliver oxygen to the tissues. 3-diphosphoglycerate (3-DPG) being the major portion of intracellular organic phosphates has received great attention in the last few years. Dubin et al (5) found normal red cell 3-DPG in rats after administration of triiodothyronine. Erythrocytes of healthy persons incubated with thyroid hormone were found by Snyder et al (15) and Miller et al (11) to have increased 3-DPG levels. Ruegel et al (14) made the observation in 1 healthy newborn infant that the red cell 3-DPG and plasma thyroxine level gradually increased during the first 4 days of life and that thereafter both gradually decreased.

Astrup (1) found all values within normal limits for oxygen affinity of Hb and red cell 3-DPG in 4 hyperthyroid patients. Miller (13) reported in patients with hyperthyroidism who had diminished affinity of Hb for oxygen and increased red cell 3-DPG. Eight hyperthyroid patients were found by Miller et al (11) to have elevated red cell 3-DPG. The same authors suggested that 3-DPG may be used to follow clinically remission and exacerbation in the Graves disease state. Vallet et al (18) observed elevated red cell 3-DPG in hyperthyroid patients but of significantly lower degree than previous authors when correct on was made for the hematocrit level.

In the present work an attempt has been made to follow the red cell 3-DPG level in hyperthyroid patients before, during and after treatment when the patients had become clinically euthyroid. The aim has been to find out whether the measurement could be used as a parameter evaluating the result of treatment. The effect of smoking on the 3-DPG level has been investigated.

MATERIAL

Twenty-eight hyperthyroid patients were investigated: 4 women and 4 men. Their ages varied from 13 to 77 years (mean 48). A control group included 4 healthy volunteers: 11 women and 13 men aged 16-45 years (mean 28).

The clinical diagnosis was confirmed by laboratory determinations (Table I). FBJ was low in all patients and T₄ in all but one. T₃ uptake was determined in one patient and was found to be elevated in 19 of them. Standard bicarbonate was within normal limits in 8 patients, Hb concentration was slightly below or at the lower borderline in 7 patients.

Table 1 *Clinical and laboratory data of 28 hyperthyroid patients before treatment*

P. L. no.	Sex	Age (y)	PBJ ($\mu\text{g}/100\text{ ml}$)	T ($^{\circ}\text{C}$)	Uptake (%)	3-DPG (mmol/l RBC)	Hb (g/100 ml)	Standard bicarbonate (mEq/l)
1	♀	63	13.5	39.9	High	6.61	11.3	5.0
	♀	62	19.8	38	High	6.54	11.2	4.0
3	♀	38	11.0	34	High	6.37	12.6	5.0
4	♀	65	11.9	35.1	Normal	6.18	11.5	23.0
5	♂	37	13.7	34.1	High	6.16	13.3	22.0
6	♀	79	18.0	30.6	High	6.16	12.2	4.5
7	♀	59	14.9	35.1	High	6.08	11.5	23.0
8	♀	35	14.5	32.9	High	6.04	12.9	19.5
9	♀	73	9.0	34.0	High	6.02	11.2	4.5
10	♀	72	11.5	33.6	High	5.98	12.8	4.5
11	♀	35	12.1	34.8	High	5.97	12.8	23.5
12	♀	79	9.8	33.3	—	5.71	13.2	1.5
13	♀	59	11	34.9	High	5.70	12.7	24.5
14	♀	34	12.0	34.0	—	5.59	13.0	25.0
15	♀	4	1.0	36.8	—	5.55	11.7	4.0
16	♀	83	11.6	34.5	Normal	5.38	12.7	4.5
17	♀	26	1.3	36.1	High	5.29	12.6	25.5
18	♀	39	8	34.3	High	5.24	13.7	22.0
19	♀	62	11.5	34.3	—	5.19	13.3	5.0
20	♀	76	12.6	33.7	High	4.95	13.0	22.0
21	♀	18	11.9	34.0	—	4.91	14.1	23.0
22	♂	67	10.5	34.0	High	4.83	14.7	4.5
23	♀	67	16.8	33.7	High	4.82	11.7	23.5
24	♀	42	11.9	33.9	High	4.78	12.9	4.5
25	♂	13	17.0	32.0	—	4.77	12.7	20.0
26	♂	51	13.9	34.4	High	4.71	15.4	25.5
27	♀	33	7.5	33.2	High	4.58	13.3	22.0
28	♀	77	1	30.1	Normal	4.39	13.1	22.5
Mean values								
Hyperthyroid 48			12.57 ± 2.8 (S.D.)	35.9 ± 3.4 (S.D.)		5.51 ± 0.6 (S.D.)		
Normal			4.5 ± 3	$30-37.0$		4.88 ± 0.4 (S.D.)	$11.6-16.6$	$19-26$

METHODS

Vein blood was drawn from the antecubital fossa into 10 ml Vacutainer tubes containing 143 USP unit sodium heparin. Within 1 min 1 ml blood was placed in 4 ml chilled perchloric acid and homogenized and extracted. The extracts were neutralized with potassium bicarbonate and used for 2,3-DPG by the enzymatic method of Eriksson and de Verdier (7). All analyses were performed in duplicate.

Hb, Hct, acid base balance, PBJ, T, and Uptake were determined by standard methods. Student's *t*-test was used for statistical analysis.

RESULTS

Fifteen of 28 hyperthyroid patients showed elevation of 3-DPG above the normal range (Fig. 1). The mean 3-DPG value for the normal group was 4.88 ± 0.4 mmol/l RBC. For the hyperthyroid group the mean value before treatment was 5.51 ± 0.6

mmol/l RBC. The difference between the two groups is highly significant ($p < 0.001$).

No difference was found in the level of erythrocyte 3-DPG between smokers and non-smokers. When the patients were divided into a female and a male group, a higher but statistically non-significant level ($p > 0.05$) was found in the first group.

When 7 patients with a Hb level slightly below or at the lower borderline were excluded, the 2,3-DPG elevation in the hyperthyroid group was still significantly higher than in the control group ($p < 0.005$). Taking into consideration that women have lower Hb levels than men, it could be expected that in our hyperthyroid group, composed mostly of women, the 2,3-DPG level would be higher than in the normal group with almost equal proportions of men and women. Therefore a new analysis was performed of the results in the female hyperthyroid

Table 11 Red cell 2,3-DPG, PBJ and T of 19 hyperthyroid patient before and after treatment

PBJ values (within parentheses) for patient treated with ^{131}I were excluded when mean values were calculated

Pat. No.	2,3-DPG (mmol/l RBC)		PBJ ($\mu\text{g}/100\text{ ml}$)		T (%)		Therapy
	Before	After	Before	After	Before	After	
4	6.18	5.90	10.5	5.0	160	104	Carbamazole
5	6.16	5.02	18.0	1.3	106	80	Propylthiouracil
9	6.02	5.76	9.0	(1.8)	150	78	I
10	5.98	6.84	11.5	(10...)	136	1...5	I
11	5.97	5.38	1...1	4.6	158	78	Carbamazole
1	5.71	6.49	9.8	5	133	77	Op - levothyronine
14	5.59	6.54	1.0	3.0	190	80	Op
15	5.55	4.98	1.0	4.9	168	98	Carbamazole
16	5.38	4.96	11.6	10.0	145	119	Carbamazole
17	5.29	5.64	1...3	5.3	161	117	Propylthiouracil
18	5...4	5.19	8	(3.1)	1...3	83	1...1
19	5.19	5.1	11.5	8.5	143	83	Carbamazole
1	4.91	4.98	11.5	7.5	140	114	Carbamazole levothyronine
22	4.83	4.85	10.5	(6.5)	140	79	I
23	4.87	4.35	16.8	(15.0)	197	104	I
4	4.78	5.43	11.9	(4.5)	189	88	I
26	4.71	4.47	13.9	1...9	174	78	Propylthiouracil levothyronine
27	4.48	4.38	7.5	7.5	137	130	Carbamazole levothyronine
28	4.39	4.70	1	(5.8)	101	73	1...1
Mean \pm S.D.	5.33 \pm 0.6	4.35 \pm 0.7	11.73 \pm 5	6.08 \pm 3.3	157 \pm 28	94 \pm 19	

group including the patient with Hb levels at the lower borderline or below. The mean values were 5.4 ± 0.6 for 17 hyperthyroid females and 5.00 ± 0.4 for 11 normal females with Hb levels well within normal limit. The difference is non-significant ($p > 0.05$).

The mean value for red cell 2,3-DPG before and after treatment showed no significant difference whereas the corresponding values for PBJ and T showed highly significant difference ($p < 0.001$) (Table 11).

DISCUSSION

The red cell 2,3-DPG in hyperthyroid patient was significantly higher than in the controls. The magnitude of this increase was far from being of the same order as found by Miller *et al.* (11) who found elevations above the normal range in all patients investigated. In the present study only 15 of 28 patients had values above the normal range.

Red cell 2,3-DPG is elevated in hypoxic conditions (10, 11, 17, 19) and this has been interpreted as a compensatory manifestation since the oxygen transport to the tissues is improved through dis-

placement of the oxyhemoglobin curve to the right. Eaton and Brewer (6) as well as Hjelm (10) have reported an inverse correlation between 2,3-DPG and Hb concentration in healthy persons. In the

2,3-DPG

normal RBC

0.8

0.6

0.4

0.2

0

-0.2

-0.4

-0.6

-0.8

-1.0

-1.2

-1.4

-1.6

-1.8

-2.0

-2.2

-2.4

-2.6

-2.8

-3.0

-3.2

-3.4

-3.6

-3.8

-4.0

-4.2

-4.4

-4.6

-4.8

-5.0

-5.2

-5.4

-5.6

-5.8

-6.0

-6.2

-6.4

-6.6

-6.8

-7.0

-7.2

-7.4

-7.6

-7.8

-8.0

-8.2

-8.4

-8.6

-8.8

-9.0

-9.2

-9.4

-9.6

-9.8

-10.0

-10.2

-10.4

-10.6

-10.8

-11.0

-11.2

-11.4

-11.6

-11.8

-12.0

-12.2

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-13.0

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-27.0

-27.2

-27.4

-27.6

-27.8

-28.0

-28.2

-28.4

-28.6

-28.8

-29.0

-29.2

-29.4

-29.6

-29.8

-30.0

-30.2

-30.4

-30.6

-30.8

-31.0

-31.2

-31.4

-31.6

-31.8

-32.0

-32.2

-32.4

-32.6

-32.8

-33.0

-33.2

-33.4

-33.6

-33.8

-34.0

-34.2

-34.4

-34.6

-34.8

-35.0

-35.2

-35.4

-35.6

-35.8

-36.0

-36.2

-36.4

-36.6

-36.8

-37.0

-37.2

-37.4

-37.6

-37.8

-38.0

-38.2

-38.4

-38.6

-38.8

-39.0

-39.2

-39.4

-39.6

-39.8

-40.0

-40.2

-40.4

-40.6

-40.8

-41.0

-41.2

-41.4

-41.6

-41.8

-42.0

-42.2

-42.4

-42.6

-42.8

-43.0

-43.2

-43.4

-43.6

-43.8

-44.0

-44.2

-44.4

-44.6

-44.8

-45.0

-45.2

ARRHYTHMIAS IN THE EARLIEST PHASE OF ACUTE MYOCARDIAL INFARCTION

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Abstract During 4-year period acute myocardial infarctions (AMI) occurred 26 times while the patients were monitored on ECG in our Coronary Care Unit (CCU). The arrhythmias observed during the earliest phase were as follows: No or non-serious arrhythmias were seen in 11 cases, 1 of them the AMI were considered to be small. One patient with small AMI had ventricular extrasystoles (VES) bradyarrhythmia was seen in 7 cases, in 6, including all the 3 patients with inferior or posterior AMIs it occurred during the first minutes. In one with VES bradyarrhythmia developed 2 hours after the AMI. In 4 cases VES and extrinsic tachycardia developed later in the course, 1-2 no other arrhythmias were seen. Primary ventricular fibrillation was seen in 3 patients, in one as the first symptom in the others 12 and 30 min after onset. They were all defibrillated and left the hospital in good condition. Four patients developed cardiogenic shock shortly after the onset. Two died within 40 min, the others within the next few hours. Except for the agonal rhythm disturbances no arrhythmias were seen. Altogether 6 patients died and 3 more would probably have died if they had not been in the CCU when they developed the AMI. Five or more than half of these 9 patients, died or would have died within 40 min, during the usual prehospital period. This is in accordance with other studies and it poses probably one of our greatest medical problems in terms of loss of potentially salvageable life.

More than half of the deaths from coronary heart disease (CHD) occur within 1 hour of onset of symptoms (7, 6, 7, 12). It is supposed that at least half of these sudden deaths are due to arrhythmias (10). Due to the delay between onset of symptoms and admission to the Coronary Care Unit (CCU) the clinical events occurring during the earliest phase of acute myocardial infarction (AMI) in man are largely unknown. The ideal situation for studying the earliest period is when the patients are already monitored on ECG when they develop the AMIs (3, 9). During a 4 year period this happened

26 times among 25 patients in our CCU in the same period altogether 688 patients with AMIs and 243 patients with intermediate coronary syndrome were treated in the same unit.

METHODS

The patients reported were treated in the CCU at Diakonskehusets Hospital from Jan. 1970 to Jan. 1974. Three patients were still in the CCU 5 days following an AMI when they sustained a new one. The others had all had one or more non-severe but typical attack of chest pain without accompanying enzyme abnormalities, and with no or only minor ECG changes. One patient had been in the CCU for only 10 min, the others 2-40 hours when they developed an AMI. All patients in the CCU were monitored on ECG. A 4-channel oscilloscope was continuously observed and all arrhythmias were recorded. The diagnosis of AMI was based on sudden onset of typical chest pain (17) very probable or possible ECG changes (17) and SGOT >50 U/l the earliest phase all patients were treated with morphine, oxygen and verapamil. The antiarrhythmic treatment given is mentioned in the case reports.

CASE REPORTS

The patients are numbered according to their admission date and divided into groups according to the size of the AMI and to the rhythm disturbances. When nothing is mentioned they had had no known earlier AMI, had not used digitalis or diuretics and they left the hospital in good condition. Ventricular extrasystoles (VES) less than 3/min are called few.

Five male patients (nos. 8, 11, 12, 18 and 19), 51-77 years old and 4 female (nos. 10, 21, 23 and 26) 58-75 years old had signs of small AMIs. Serial ECGs showed transient ST-T changes and the SGOT values increased from 12-20 U before the AMIs to 52-110 U after. Case 10 had supraventricular tachycardia about 11 min after onset with heart rate (HR) of 160/min. It lasted for only 5 min. Case 23 had frequent supra-VES with aberrant conduction 8-10 min after the onset and of 4-6 min duration. Case 26 developed VES 3 min after onset

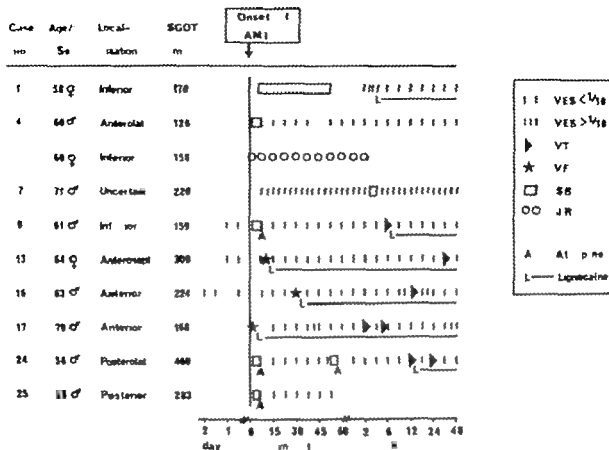


Fig 1 Arrhythmias during the first 48 hours in patients with AMI VES=ventricular extrasystoles, VT=ven-

tricular tachycardia, VF=ventricular fibrillation, SB=sinus bradycardia, JR=junctional rhythm

was treated with lignocaine for a few hours. In the other patients no arrhythmias were seen except for a few VES and no antiarrhythmic drugs were given.

Three male patients (nos. 14, 15 and 20) 61-77 years old had anterior AMI with maximal SGOT values of 125-264 U. Two of them (nos. 14 and 20) had had two earlier AMIs and they both used digitalis. All 3 had only a few VES and no antiarrhythmic treatment was given. Case 14 developed LBBB on the third day. Seven patients developed bradyarrhythmias (Fig. 1).

Case 1 developed sinus bradycardia (SB) of short duration HR 48/min about 5 min after the onset. The SB may have been caused by morphine given 1 min earlier. No atropine was given but later lignocaine because of VES.

Case 4 developed SB HR 44/min 3 min after onset. It lasted only about 5 min. Atropine was not given.

Case 5 developed junctional rhythm (JR) HR 55/min 2-3 min after onset. It lasted for about 2 hours. No other complications were seen until she sustained a new AMI on the 5th day while she was still being monitored on ECG (see case 6).

Case 7 developed his third AMI in our CCU. He was in heart failure and had multifocal VES 1-2 min after onset. He was treated with digoxin and furosemide but

not with lignocaine. Two hours later he had a short episode of SB HR 46/min. He died 3 weeks later of heart failure.

Case 9 The SB which occurred 2-3 min after onset was abolished with a single dose of atropine. He also had a few VES and a short run of ventricular tachycardia (VT) which was treated with lignocaine.

Case 24 developed SB about 2 min after onset, HR 33/min. Atropine was immediately effective but he needed a new injection 30 min later. He also developed VES and VT but clinical condition deteriorated and he died on the 4th day of cardiogenic shock.

Case 25 developed SB about 3 min after onset HR 35/min. Atropine was immediately effective.

Three patients developed ventricular fibrillation (VF) (Fig. 1). They were all defibrillated at once with 400 Ws. All 3 also had VT later in spite of lignocaine therapy. Their course was otherwise uncomplicated.

Case 13 had a few multifocal VES before as well as after an AMI. VF occurred unexpectedly 1 min after onset.

Case 16 had had two earlier AMIs. He had a few VES before as well as after an AMI. VF occurred unexpectedly 30 min after onset.

Case 17 had no pain and no arrhythmias when he

suddenly developed VF. When he woke shortly afterwards he had severe typical chest pain. Serial ECG showed unequivocal AMI.

Four patients (no. 1, female 75 y., no. 3 male 64 y., no. 6, female 60 y. and no. 12 male 73 y.) all had very serious clinical condition with hypotension and heart failure shortly after onset. They all developed cardiogenic shock and died within 30 and 40 min. 5 and 6 hours respectively. Except for the apical rhythm disturbances no arrhythmias were seen.

The events occurring at different intervals after the onset of the AMIs are summed up below.

(1) During the first 30 min. Four patients, of whom one died, developed cardiogenic shock. Three patients had VF, all were defibrillated. Two patients of whom one was in heart failure had multiple VES. Six patients developed bradycardias. 5 SB and one JR. 1 one it caused spontaneously in 3 after atropine injection, while in 1 it continued without complications. Among the remaining 11 patients one had short attack of supra-ventricular tachycardia, the others only few VES or no arrhythmias at all.

(2) During the next 30 min. Another patient in cardiogenic shock died and one had a new short attack of SB.

(3) During the following 47 hours (1–48 hours after onset) The remaining patients in cardiogenic shock died. All the 3 patients with prior VF had VT although they were on lignocaine therapy. Among 3 of the patients with prior SB, had VT and one multiple VES. Sixteen patients including all the 11 without prior serious arrhythmias, had only few VES or no arrhythmias at all.

(4) During the period from the 3rd day until the patients left the hospital. One patient with prior SB and VT died in cardiogenic shock. One patient with intractable heart failure died. One patient had re-infarction. Among the other patients one developed LBBB. The 3 patients with prior VF had VES whereas none of the other patients had serious arrhythmias.

DISCUSSION

Only a few patients sustain their AMIs while they are being monitored on ECG in a CCU. These patients have usually been admitted to the CCU because an AMI could not be excluded until serial ECG and laboratory tests were available. Meanwhile they develop an AMI. A few patients may also have a re-infarction while they are still being monitored because of the first one. In our CCU less than 4% of the patients with AMI had it while they were being monitored on ECG.

The earliest event during the AMI probably depends upon the size of the AMI (5, 15). In the present material of 26 AMIs 9 were considered to be small. This is a higher incidence of small AMI than in our total AMI material (15) and may

plain the high incidence of patients without serious arrhythmias. In 4 patients however the clinical courses were very serious. It is remarkable that none of these patients had arrhythmias.

Primary VF is a very early complication in AMI and is seldom preceded by warning arrhythmias (1, 8). Three of our patients were probably saved because they were in the CCU when they developed VF. In one of the patients VF was the first symptom of AMI. In the other two it occurred without convincing warning arrhythmias 1 and 30 min after onset.

Bradycardias are also seen with a higher incidence the earlier the patients are observed (1) especially in patients with inferior or posterior AMIs. In all our 5 patients with this localization and in one patient with anterior AMI the bradycardias occurred during the first minutes in only one patient more than one hour after onset.

The number of cases in the present study is too small to draw any certain conclusions. The true variation of the earliest clinical course in AMI may be another. Our experiences are however in accordance with other studies where more than half of the CHD deaths occurred during the first hour (1, 6, 7, 11) and where a high percentage of these deaths were due to primary VF (1). If our 25 patients with 26 AMIs had been admitted to the CCU one hour after the attack, 5 patients would probably have been dead before they reached the hospital. Two of these patients died from cardiogenic shock, the other 3 would have died from primary VF. Four patients died later in the hospital. Altogether 8 patients would have died of whom 5 within one hour.

The prehospital phase of AMI poses probably the greatest single medical problem in terms of loss of potentially salvageable life (4). Even when mobile CCUs are available only 6% of the patients with AMI are seen within 30 min after onset (1). Among patients dying outside the hospital 78% died before ambulance was called (13). The potential profit of prehospital premonitory care station in order to observe patients who display warning symptoms would also be limited by the fact that only two-thirds of patients with AMI have prodromata (14) and that among patients dying outside hospital only one-third thought these prodromata were serious enough to seek medical consultation (15).

Establishment of both mobile CCU and pre-

coronary units together with better education of physicians and patients especially in the highest risk groups is probably the best answer to this problem today.

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PRE EXCITATION IN YOUNG MEN

Incidence, some Anthropometric Data and Physical Work Capacity

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Abstract Screened young men with pre-excitation found at an enlistment medical examination of conscript (giving an incidence of 1/1000) have been compared with 11 randomly selected healthy men of the same age. None in the pre-excitation group had any signs of organic heart disease or history of tachycardial bout. Those with pre-excitation were significantly shorter and had a slightly higher BP than the controls, but did not differ regarding physical work capacity (W_{170}) or heart volume (HV). No difference was found in these respects between types A and B pre-excitation. W_{170} was, when adjusted for differences in HV, significantly higher in 8 subjects, whose ECG were totally or partially normalized during exercise than in 8 subjects, whom no normalization occurred.

particularly as the correct timing between atrial and ventricular systole is in part impaired so that the isometric contraction of one of the ventricles starts before the atrial mechanical systole has ended. This might give a decrease in the stroke volume, at least at higher heart rates.

The aim of our study has been to determine the incidence of pre-excitation in a general population and the frequency of concomitant bouts of tachycardia and other disorders and to evaluate the consequence of pre-excitation to physical work capacity.

It is nowadays generally agreed that there is an anomalous conduction bypassing the atrioventricular node, patients with pre-excitation. It is often associated with bouts of tachycardia (4, 8, 10, 16, 20, 22, 26). Pre-excitation is reported to be more common in congenital heart disease such as cardiomyopathy, subvalvular aortic stenosis and Ebstein's anomaly of the right ventricle (5, 7, 8, 11, 13, 20, 25, 26) and some congenital cerebral disorders (4, 5, 11, 20, 21). Of subjects with pre-excitation found in a hospital population 35-80% are reported to have complicating disease (4, 20). The functional effect of pre-excitation has been studied by right heart catheterization and other methods and a impairment of ejection in one or both ventricles has been claimed (1, 3, 13, 15, 17, 18, 23, 26). In view of the connection with different disorders of the heart a diminished capacity for physical exercise may be expected,

MATERIAL AND METHODS

Altogether 16 men with pre-excitation were observed in a material of 16000 conscripts, about 18 years of age, consistently examined during the course of 18 months at the Enlistment Centre Solna. From the same material 112 healthy conscript with ordinary ECG were randomly selected and used as control material. A criterion of pre-excitation a S-wave in at least in leads II and with a duration of 3 msec in at least one of them was used (17). The P-Q interval turned out to be less than 12 msec in all cases. The ECG was classified as type A when the R wave was the sole or the largest deflection in the right precordial leads, particularly CR and CR₁. If the sole or largest deflection in the right precordial leads was an S or QS wave, the ECG was classified as type B.

In both groups heart volume (HV), the supine and upright positions was estimated (11). An orthostatic test was done including heart rate (HR), BP and ECG after standing for 8 min. The exercise test was performed on the sitting position on an electro-dynamically braked cycle ergometer (Siemens-Elmex, EM 370-1) with stepwise increasing load every 6th min up to a HR of about 170 beats/min (4). The load giving a HR of 170 beats/min (W_{170}) was used as a measure of physical work capacity. The ECG was recorded with an ml.

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Table 1 Some anthropometric and other data in 112 controls and 16 subjects with pre-excitation

	Control		Pre-excitation		<i>p</i> <
	Mean	S.D.	Mean	S.D.	
Height (cm)	179.9	6.5	176.3	6.4	0.05
Weight (kg)	68.3	10.0	67.7	7.8	(0.90)
HR at rest (beats/min)	66	11	69	11	(0.40)
HR in orthostatic test (beats/min)	85	13	85	1	(1)
Blood volume (l)	5.6	0.7	5.1	0.7	0.05
Systolic BP recumbent (mmHg)	123.3	11.3	130.9	13.0	(0.10)
Diastolic BP recumbent (mmHg)	73.9	8.0	78.8	6.2	0.05
Systolic BP standing (mmHg)	125.1	10.1	130.3	15.1	(0.10)
Diastolic BP standing (mmHg)	81.1	7.9	81.9	7.9	(1)
W_{max} (kpm/min)	1033	19	1021	234	(0.90)
HV (ml)	771	118	779	108	(0.80)

jet electrocardiograph (Mingograph III Siemens-Elema). In repose CR lead were used and during exercise chest-lead leads (V₁) BP was measured with a calibrated cuff on the right upper arm by auscultation. The observed value was rounded off to the nearest level of 5 mmHg. The significance of differences between groups was assessed using the *t*-distribution.

RESULTS

Sixteen cases of pre-excitation were found: 5 of type A and 11 of type B giving an incidence of 1/1000 in the population consisting of 18-year-old men. They had no knowledge of their abnormality. None of them had any history of tachycardial bouts and physical examination revealed no sign of cardiac disease besides the pre-excitation. In the pre-excitation group the mean height was 3.6 cm shorter than in the control group ($p < 0.05$) (Table 1). The blood volume was on an average 0.5 l smaller (mean 5.1 l, S.D. 0.7) in the pre-excitation than in the control group ($p < 0.05$). The resting diastolic BP in the recumbent position in the pre-excitation group was 78.8 mmHg (S.D. 6.2) which was 4.9 mmHg higher than in the control group ($p < 0.05$). Systolic BP was numerically higher in the

pre-excitation group than in the control: 5.6 mmHg ($p < 0.10$) in recumbent and 5.2 mmHg ($p < 0.10$) in standing position.

The mean values for W_{max} and HV in the pre-excitation group did not differ from those in the control group (Table 1). During the orthostatic test there was no change of the degree of pre-excitation and no difference in orthostatic reaction regarding BP and HR as compared to the controls (Table 1). There was no difference between pre-excitation type A and type B regarding W_{max} and HV (Table II).

During exercise three subjects with type A and five with type B pre-excitation had a partial or total normalization of their ECG. Those with a regress of the pre-excitation signs had a broader S wave at rest than those with unchanged degree of pre-excitation during exercise (4.5 and 4.3 msec respectively) but the difference was not significant. There was a tendency to a higher physical work capacity for cases with regress in pre-excitation during exercise than for cases without but the difference (168 kpm/min) was not significant (Table III). No significant difference in HV was found. The tendency to higher work capacity

Table II Physical work capacity and heart volume in types A and B pre-excitation

	N	\bar{x}	S.D.	<i>p</i>		N	\bar{x}	S.D.	<i>p</i>
W_{max} (kpm/min)					W_{max} (kpm/min)				
A	5	1080	144	<0.7	Regress	8	1106	195	<0.70
B	11	995	268		No regress	8	918	232	
HV (ml)					HV (ml)				
A	5	746	43	<0.5	Regress	8	768	105	<0.70
B	11	794	126		No regress	8	791	116	

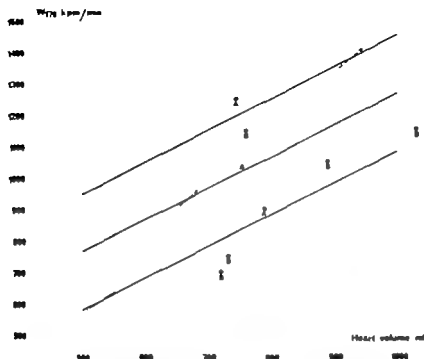


Fig. 1 W_{170} relation to HV in 11 normals. Regression line ± 5 E.M. ($W_{170} = 0.98 \times HV + 779.3$ S.E.M. = 186.7 = 0.53 $p < 0.001$) = regression for subjects with pre-excitation during exercise (O) — regression for subject with no pre-excitation (●) A and B types of pre-excitation

At a HR of 170 beats/min for cases with pre-excitation during exercise was found to be significant ($p < 0.05$) when differences in HV had been accounted for by means of analysis of covariance. I.e. those with a pre-excitation had a higher physical work capacity at a HR of 170 beats/min in relation to HV (Fig. 1). The difference between adjusted means, i.e. the critical distance between parallel regression line was 703 kpm/min.

DISCUSSION

The incidence of pre-excitation in the population studied by us 1/1000 is rather low in relation to other reports of incidence ranging from 1.6 to 3.1/1000 in similar non-hospital populations mainly consisting of fairly young men (6, 8, 22). None in our material had any history of tachycardial bouts which agrees with earlier reports that the frequency of tachycardias in pre-excitation 0–4% is low in general health surveys (16, 22). However frequencies as high as 1% have been reported () and in hospital ECG surveys the figure is considered to be 50–70% (4, 10, 16, 20, 26). The frequency of organic heart disease in pre-excitation in this study was low (zero) which is in agreement with reports from other non-hospital populations (2, 14, 16, 22).

The physical work capacity was normal in the present material of pre-excitation which seems to indicate that pre-excitation per se is fairly harmless. A study of physical work capacity in pre-excitation has earlier been made only by Sandberg (19) who found a moderate reduction. The difference can be explained by the fact that his pre-excitation cases were older and more heterogeneous and belonged to a hospital population aware of their anomaly. Furthermore his control group was younger than the patient group. It is however probable that pre-excitation may have mechanical consequences at higher HR during exercise since it was found in this study that there is a significant difference in physical work capacity between those in whom the pre-excitation diminished or disappeared during exercise and those in whom it remained unchanged or became worse.

We have no explanation to offer at present regarding the difference in body height and BP in comparison to the control group.

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HEART FAILURE AS COMPLICATION OF LITHIUM TREATMENT

Preliminary Report of a Fatal Case

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Lithium has been used in the treatment of psychiatric disorders since 1949 (1) but as early as in 1907 there was warning (2). The Practitioner (3) against depression and dilatation of the heart. Few cardiac side effects have since been reported. Temporary ECG changes with flattening of the T waves have been described (3-6) as have frequent sinusoidal extrasystoles in association with therapeutic concentrations (10). One fatal case of interstitial myocarditis has been described by Tazewell in 1971 (12). The lithium determinations in the latter case have been criticized by Schön (5). We report a fatality probably due to prolonged lithium treatment.

CASE REPORT

A 46-year-old woman with long history of episodic depressions. She had suffered from slight asthma for several years and, because of this had taken potassium iodide 0.3 g/day since 1968. Lithium treatment was started in April 1971. Monthly serum assays showed Li^+ levels of 0.7-1.1 mEq/L. She was simultaneously treated with amipropazine 75 mg/day. In March 1973 she was admitted to hospital because of weight gain of 25 kg over the past 3 years to 89 kg. She complained of swelling, constipation, chilliness and inactivity. Lithium had been withdrawn one week prior to admission.

Physical findings included obesity, periorbital oedema and bourness. The thyroid gland was not enlarged. Regular tachycardia with fast sounds and no murmurs. BP 120/80. Laboratory findings: ESR 8 mm, Hb 12.7 g/100 ml, cholesterol 360 mg/100 ml, TG 115 mg/100 ml, FBS 105 mg/dl, T 3 70% (normal 80-170), BMR -15%, TSH 58 μ U/ml (normal <10), ^{125}I uptake in 24 h as 3%. Serum electrophoresis normal. No antibodies against TBG or thyroid cytoplasm.

Substitution therapy was started with thyroxine 0.1 mg/day. One week later addition of triiodothyronine 60 μ g/day for 14 days. After 1 month therapy with thyroxine up to 0.25 mg/day ESR was 3 mm, cholesterol 91 mg/100 ml, BMR -22%, T 3 99% and TSH <3 μ U/ml. Weight loss of 8 kg. Serology against Coxsackie virus B negative.

On admission X-ray of the heart revealed marked

general enlargement to 180 ml/m² BSA and slight right-sided pleural effusion. ECG showed sinus tachycardia (100/min), low voltage in the extremity leads and generally flat T waves. No arrhythmia during the whole treatment period. After 4 weeks of thyroxine substitution the heart size was unchanged and there was more fluid in the right pleural cavity. Echocardiography did not disclose any pericardial effusion. ECG now showed higher amplitudes but was otherwise unchanged. Thoracentesis on the right side yielded 780 ml fluid.

Digitalis was not given, as the heart failure was attributed to hypothyroidism and the patient showed no clinical sign of decompensation. However in spite of adequate hormone substitution cardiac decompensation developed and after 5 weeks of thyroxine treatment the patient began to complain of discomfort, especially of dyspnoea. Because of increasing cardiac insufficiency the patient was transferred after 7 weeks to the Department of Endocrinology and Cardiology of Sahlgrenska Hospital, Gothenburg. An idiopathic urticaria/urticaria was diagnosed. The patient was treated with digitalis and diuretics and her symptoms improved. A thrombosis in the right subclavian vein was treated with heparin and dicoumarol. The patient was discharged after 3 1/2 months of hormone treatment, on maintenance with digoxin 0.25 mg 1, furosemide 40 mg 2, spironolactone 25 mg 4, thyroxine 0.15 mg 1 and dicoumarol. She died suddenly 3 weeks later.

Autopsy findings

Autopsy was performed 40 hours after death. The patient was 177 cm tall and weighed 66 kg.

The heart weighed 280 g and showed slight hypertrophy of both the left and right chambers. The myocardium was somewhat streaked and granular. In the coronary arteries there were only few scattered non-calcified intimal deposits in the intima. The bronchi were not narrowed.

Microscopic examination revealed small scars in number of regions in the myocardium, these consisted of fairly acellular connective tissue. There were also partially acellulose regions of oedematous connective tissue containing sparse, or in places denser, collection of inflammatory cells, especially mononuclear cells but also some neutrophilic granulocytes and an occa-

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TRANSVENOUS CARDIAC PACEMAKER AS A FOCUS OF SALMONELLA INFECTION IN A PATIENT WITH HEART BLOCK

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Abstract. A case of *S. typhimurium* septicemia in association with permanently implanted transvenous pacemaker is described. The *Salmonella* infection had probably been contracted long before and was possibly the cause of failure in heart conduction. Antibiotic treatment alone was without result, but after removal of the pacemaker system it resulted in eradication of the *Salmonella* infection and was also followed by elimination of the heart block. Three years after the operation there is still no need for artificial pacing.

Foreign material intracardially implanted is a factor predisposing for severe infections. Thus septicemia and endocarditis have been reported in patients with prosthetic cardiac valves (11) and with intracardiac catheters for the alleviation of hydrocephalus (18). There are also some reports of septicemia (8, 9) and endocarditis (5, 6, 17) in patients with permanent transvenous pacemakers, which since 1958 are commonly used for artificial pacing (7). The bacteria most often involved have been Gram-positive cocci (5).

This report deals with a *Salmonella* infection in a transvenous cardiac pacemaker system, resulting in septicemia. Combined conservative and surgical treatment resulted not only in elimination of the *Salmonella* infection but was also followed by disappearance of a 2nd-3rd degree heart block, which had been the reason for pacemaker implantation.

CASE REPORT

Clinical data

A 56-year-old man who had experienced an attack of high fever of unknown origin in 1935 when serving in S.edish naval station. At the same time several of his fellows had similar febrile illnesses, with fatal outcome

in two. Since then the patient has had about twice yearly episodes of fever up to or above 40°C, typically subsiding within 3 days and without any concomitant symptoms. While the history is vague concerning the 40° attacks have been better recorded during the last two decades.

He had made some trips to Mediterranean countries and the Canaries during the last 10 years, but on these occasions he had no symptoms of gastroenteritis compatible with salmonellosis. In 1953 he suffered from repeated stomach ulcers, which led to partial gastric resection. ECG has been normal and no signs or symptoms of cardiovascular diseases or hypertension have been noted.

On May 1 and 2, 1968 repeated Adams Stokes episodes occurred and the ECG indicated an atrioventricular block changing between 2nd and 3rd degree. A transvenous cardiac electrode was inserted and attached to an external pacemaker. Regular sinus rhythm was established and the patient was discharged from the Thoracic Clinic one week later in good state of health. There were no elevations of serum transaminases and heart volume was normal according to X-ray.

In August 1968 subcutaneous implantation of the pacemaker into the left abdominal wall was performed. Because of a purulent skin erosion in the right parasternal area, caused by a too superficial position of the electrode, dissection of it to a more profound layer had to be made. Though no pathogenic bacteria could be cultivated from the erosion, the surgical intervention was combined with ampicillin treatment. Some irritation of the skin persisted during the following months and there was also tenderness over the pacemaker pocket. No bacterial cultures were performed. In Dec. 1969 he had chills and dizziness of the same type as before but no signs of skin infection.

In Feb. 1970 the pacemaker had been dislodged upwards in the left abdominal wall and was replaced by a new one implanted in a lower region of the left abdominal wall. There were no signs of infection and cultures from the pacemaker pocket yielded no growth. From the autumn 1970 there were continuously erosions along the electrode and infection with *S. aureus* and *E. coli* was found. On Oct. 27, 1970 for

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the first time there was growth from the lesions of *Salmonella typhimurium* phage type 1. Stool cultures were negative for *Salmonella* but the Widal test was positive against *S. typhimurium* O and H antigens.

He was again admitted to the Thoracic Clinic at the beginning of Dec. There was obviously a persistent infection along the whole subcutaneous part of the electrode from the right supraclavicular fossa to the pacemaker box just above the left groin. Two erosions with purulent secretion again yielded growth of *S. typhimurium*. A revision of the erosions was performed and, in a later session the electrode was in a deeper location. Ampicillin was given and he was discharged after about one week. Two weeks later the patient had a new episode of high fever and persistent infected skin erosion. As it did not seem possible to eliminate the *Salmonella* infection without a radical revision he was again hospitalized. On Dec. 30 the pacemaker system was partly removed and a temporary bipolar electrode was inserted through the left cephalic vein with the tip implanted in the apex of the right ventricle and connected to an external pacemaker. Blood cultures, now performed for the first time, yielded *S. typhimurium*. Such was also found in the wounds and from the drainage after operation. For this reason the patient was referred to the Department of Infectious Diseases, Rostagstråll Hospital.

On admission the patient was afebrile though not antibiotically treated but had some mosquito-like efflorescences over the chest. Cultures from blood and wound again revealed growth of *S. typhimurium*.

Treatment

The patient was treated for 3 weeks with chloramphenicol, 3.0 g daily and the temperature returned to normal. However 4 days after discontinuing the antibiotic treatment he had a fever spike up to 39.8°C with other symptoms. Three of 4 blood cultures on the day again showed growth of *S. typhimurium*. Ampicillin was given 1 week later 12 g daily. The fever subsided within 4 days. After 3 weeks the treatment was continued orally 6 g daily. This treatment was continued for altogether 3 months.

The intracardiac part of the old catheter had been left in place because of its firm attachment to the endocardium and the risk of damage of the tricuspid valve by its removal. Six weeks after admission it was, however removed without any complication except brief chills. This time blood cultures showed no growth but *S. typhimurium* was isolated from the intracardiac electrode tip and from the operation wound. Five weeks later it was obvious that the temporary pacemaker was no longer functioning and the whole system was removed. The subsequent course was quite uneventful.

Repeated blood, stool and urine cultures were negative for *Salmonella*, but the Widal titres remained high. Heart volume, as judged by X-ray, remained normal. Except for an episode of frequent ventricular extrasystoles or mixed beats on one occasion, repeated ECGs were normal. The patient was discharged 2 months after admission, still on oral ampicillin therapy.

During ampicillin treatment the serum concentration

of the drug was repeatedly recorded. It was at least 4 µg/ml and thus exceeded the minimum inhibiting concentration for the strain almost tenfold.

Follow-up examination

After discontinuation of the ampicillin treatment the patient was readmitted several times for control. The last one 3 years after discharge. The general condition has been excellent. The fever bouts have disappeared and no cardiac discomfort has been reported. X-ray of the heart has remained normal, as well as repeated ECGs. Exercise ECG immediately after discharge was normal with normal physical working capacity and there has been no need for a pacemaker. Blood cultures have remained sterile and Widal reaction has given titres as shown in Table 1. The first test was performed in another laboratory than the following ones. The result, with different values, was noted immediately after incubation and after standing in the refrigerator overnight.

DISCUSSION

The *Salmonella* infection in our patient may have been of recent origin, acquired in hospital or contracted during a trip abroad in the last decade although no enteric or other symptoms compatible with an acute salmonellosis occurred. It is also possible that the infection started as early as in 1935 when the patient suffered from an undoubtedly epidemic febrile disease. The fever periods recurring ever since 1935 but disappearing after prolonged antibiotic therapy and surgical treatment are in agreement with this theory. Another support for this theory might be the slow decrease of the high agglutination titres against O and H antigens which indicate an infection of very long duration.

Long-standing *Salmonella* infection has previously been reported by Borczyk and Lagerlöf (4). Prolonged courses of salmonellosis believed to be due to a decrease of the activity of the reticuloendothelial system in *Schistosoma mansoni* infection have also been observed (10-13). Chronic salmonellosis in a patient with impaired immune defence function has been described by Stiles et al. (19). In our patient as well as in Böttiger and Lagerlöf's there were no factors known to interfere with resistance to bacterial infection. Infection may take place at the pacemaker implantation but may also be endogenous as reported by Schwenkerdt et al. (16). It is possible that the insertion of the intracardiac electrode in our patient converted a latent chronic infection with repeated febrile episodes into a manifest septicemia.

No foci were found in the gastrointestinal or

Table 1 Bacteriological and serological findings, surgical interventions and antibiotic treatment

B=blood, W=wound D=drainage EI=electrode PM=pacemaker E=external I=internal sc.=subcutaneous
 Amp.=ampicillin Clox.=cloxacillin, Chlor.=chloramphenicol

	Growth of <i>S. typhimurium</i>				Widal test				Surgical management	Antibiotic treatment
	B	W	D	EI	O	H	1	2 H.		
1968 May										
Aug. 16									EPM	
Aug. 20									IPM Dissection of the sc. electrode	Amp
1970 Feb 13									Change of site of PM to	Amp discontinued
Oct 27		+			800	-		800		
Nov 2										
Dec 7									Revision of the erosion	Clox. Clox. discontinued
Dec 10									Dissection of the sc. electrode	Amp
Dec 11									Temporary bipolar I PM + partial removal of the old PM system	Amp discontinued
Dec 14		+								
Dec. 30	+		+							
1971 Jan. 2		+	+							
Jan. 3										
Jan. 4		+	+		400	50		400		Chlor
Jan 8		+								
Jan 17										
Jan. 18					400	1 600		800		Chlor
Jan 1		+								
Jan III		+								
Jan. 26					1 600	400		800		
Jan. 29										
Feb 8					800	1 600		400		
Feb 1					400	200		400		Amp
Feb. 17		+		+					Removal of the old intra-cardiac electrode	
Feb 23					400	800		200	Total removal of the PM system	
March 26										
April 16?					800	800		400		
May 25					400	200		100		Amp disc
1977 Oct. 10					400	400		100		
1973 March 6					400	400		100		
Sept 5					80	80		80		

Inverted values. Questionable values.

primary tract, nor as judged from the case history and X ray from the biliary tract. Unfortunately no cultures were performed from duodenal secretion. The possibility that previous ampicillin treatment had prevented the flooding of *Salmonella* in fecal samples cannot be excluded. However bacteria may dwell in the regional lymph nodes and be transported to the blood stream, as was suggested by Böttiger and Lagerlöf.

An interesting question in this case is the cause of the acute cardiac conduction failure since the patient had no history or signs of previous cardiovascular disease. Though signs of acute myocarditis were not observed the conduction defect

might have been a sequel of a preexisting myocarditis. Bengtsson and Lamberger in their 5-year follow-up study of cases suggestive of acute myocarditis (3) reported cases, though comparatively rare with atrioventricular conduction disturbances after streptococcal infections.

Acute myocarditis may appear in association with infectious diseases of various etiology. However it is a rare complication in *Salmonella* infection other than with *S. typhi* and *S. dysenteriae*. A or B. Saphra and Winer (15) reported clinical manifestations in six *Salmonella* infections in 1941-53. *Salmonella* infections in 1941-53 a was mentioned in a case.

lected during the *S. typhimurium* outbreak in Sweden in 1953 (?) ECG abnormalities were recorded in 59 cases at least 15 of them probably due to acute myocarditis. In one case ECG changes compatible with heart block were recorded. The diagnosis of myocarditis was verified at autopsy in 5 of 11 fatal cases. In 1964 Sanders and Misanik (14) described a case of acute myocarditis stemming from an overwhelming septic infection with *S. choleraesuis* and resulting in death due to rupture of the right ventricular wall. Bayer et al (1) reported a mitral subvalvular endocarditis with an abscess in the valvular ring, also caused by *S. choleraesuis*.

Spontaneous restitution of defective atrioventricular conduction may occur for instance after improvement of coronary perfusion brought about by artificial pacing as seen in cases of myocardial infarction. In our patient 3 years after removal of the pacemaker system there is still no need for artificial pacing as judged by the patient's history and repeated ECGs. This may be the result of elimination of the *Salmonella* infection followed by healing of a supposed myocarditis which had once probably caused the heart block.

Treatment with chloramphenicol and later ampicillin, unfortunately only the latter guided by determination both of bacterial sensitivity and anti-concentration, failed to cure the infection as the pacemaker system was not removed. The removal of the pacemaker generator and a subcutaneous part of the old intracardiac electrode was ineffective. As elimination of an infection is unlikely in the presence of an implanted foreign body (11, 18) we found it mandatory in spite of the risk involved (17) to remove the intracardiac part of the old catheter. The removal was justified when it was found that the catheter and the operation wound harboured *Salmonella* and more so when a definite cure and elimination of the heart block were achieved with no subsequent need for a pacemaker.

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THE FUNCTIONAL PATTERN OF THE CADAVERIC KIDNEY IN THE EARLY POSTTRANSPLANT PERIOD

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Abstract Among a series of 96 cadaveric renal transplant recipients the graft function has been studied in 25 grafts characterized by good initial function: creatinine clearances varying from 8 to 65 ml/min during the first postoperative day. Initial function did not correlate to the donor's age or to the length of warm and cold ischemia time. Mean graft function as measured by ^{51}Cr -ethylenediamine and ^{125}I -hippuran clearances during the period studied was significantly lower than that in recipients of kidneys from living donors whose ischemia times were short and well defined. During the period studied the graft function increased independently of donor's age and length of ischemia time. Connection with ischemia and rejection changes were seen in the functional pattern. The significant reduction in filtration fraction

the present study. Donors varied in age from 10 to 57 years (Table I). Twenty-two donors died of cerebral lesions caused by trauma, bleeding aneurysms or vascular occlusion. In two cases cranial trauma was accompanied by multiple traumatic lesions. Among the remaining three donors two died of brain tumors and one of herpes simplex encephalitis. All donors had been hospitalized for at least 4 hours before nephrectomy; most of them in neurosurgical ward. In 22 cases information on BP was available during the last 4 hours before nephrectomy. In three the BP was at times 60 mmHg and varied between 60 and 100 mmHg. In the remaining 19 BPs less than 70 mmHg were not registered. All had normal serum creatinine before transplantation and, in 22 urinary production and normal urinary sediment were retained up to the point of nephrectomy. In three cases information is not available on urinary production. Data with regard to the use of pressor agents are incomplete. In most cases perfusion with Perfadox was employed. Mechanical perfusion was not used.

The study material included 4 recipients: 13 men and 11 women, aged — 49 years (Table I). One patient (T 153) to transplants were performed at an interval of 8 months. Six patients were hypertensive before transplantation and six received antihypertensive therapy (Table I). Before or in connection with transplantation bilateral nephrectomy was carried out in three patients (T 118, 156 and 178) and unilateral nephrectomy in two (T 141 and 154). The remaining patients retained their own kidneys in situ during the study. In addition to the two bilateral nephrectomized patients, three patients were anuric. Among the remaining 18 six had creatinine clearance between 1 and 5 ml/min. Additional 11 patients had retained hear rates less than 3 ml/min. Sixteen of the 4 patients were on regular hemodialysis twice 10 hours weekly before transplantation.

Just after establishment of vascular anastomoses 200 mg mannitol were infused. Urinary production as observed immediately and graft function, as determined by creatinine clearance, varied from 8 to 65 ml/min during the first day (Table I). Immunosuppression consisted of azathioprine and prednisone. Immediately before transplantation azathioprine was given in a dose

In a previous study (7) we described functional patterns in renal transplants from living donors. Various factors differentiate the above situation from that seen in cadaveric transplants. The ischemic period in the latter is much longer and several aspects of the donor's condition may influence the renal function (7, 3, 9, 15). In addition histocompatibility is usually poorer than in living donor transplantation (14).

It is well known that a minority of transplanted cadaveric kidneys resume function immediately after transplantation. The present study was limited to cadaveric kidneys functioning immediately after reestablishment of blood supply to the kidney.

MATERIAL

During the period of study April 1969–June 1971 123 transplants were performed in 112 patients. Twenty-seven transplants came from living donors and 96 were cadaveric kidneys. In 25 cadaveric kidneys (26%) the initial function was good and these were included in

Table 1 Data on the donors and recipients
 C_{Cr} = creatinine clearance C_{InH_2O} = inulin clearance C_{InA} = urea clearance PP = filtration fraction

Donors			Recipients		Pretransplant		Posttransplant (day 0-1)		PP				
Age (y)	Ischemia time (min)	T no	Age (y)	C_{Cr} (ml/min)	Des-lys	BP	Anti-hypertensive treatment	Proteinuria (g/24 h)	C_{In} (ml/min)	C_{Cr} (ml/min)	C_{InH_2O} (ml/min)	C_{Cr}/C_{InH_2O}	BP
4	77	143	36	0	+	160/100	+	1	32	37	54	12.6	215/120
52	18	102	43	1	+	180/100	-	1.3	15	35	116	12.6	165/105
44	15	255	39	0	+	180/100	-	1.3	21	35	227	9.3	160/105
29	98	178	48	1	+	130/80	-	3.9	19	25	163	11.7	170/100
34	20	144	53	2.3	+	150/80	-	4.7	35	39	342	10.2	170/100
36	20	366	28	1	+	165/85	-	2.0	44	58	362	12.2	150/90
16	25	332	49	3	+	140/70	+	2.5	19	19	104	18.3	160/100
48	35	427	46		+	160/130	+	4.0	9	12	51	17.6	175/135
47	24	350	50	0	+	170/90	-	5.8	39	43	259	15.0	170/90
37	76	4.3	46	2	+	190/120	+	5.1	13	15	68	19.1	180/100
18	19	280	47	2-3	-	150/100	+	11.4	8	9	50	49.8	160/103
12	84	48	56	2.3	-	140/80	-	12.0	59	65	65	22.3	160/105
48	67	499	54	1	+	150/80	-	6.0	14	17	102	13.7	130/70
48	79	344	45	1-3	-	140/80	-	0.9	1	24	345	6.1	140/80
23	44	48	4	3-4	-	150/100	-	1.6	11	15	68	16.2	180/100
28	14	300	4	3-4	-	180/110	-	7	50	46	296	16.8	170/100
16	0	402	42	3-4	-	180/90	-	1.2	18	31	167	10.8	200/100
15	10	647	32	4-5	-	140/80	-	4.9	18	27	186	9.7	140/85
32	3	650	32	4-5	-	150/95	-	7.0	12	14	81	14.8	145/95
49	30	155	47	0	+	140/90	-	1.1	6	8	45	13.3	140/100
19	5	401	22	0	+	140/70	-	1.7	27	30	41	11.2	170/110
5	6	409	39	3-4	-	160/90	-	2.6	6	9	43	13.3	170/110
38	5	787	46	3-4	-	150/90	-	7.7	16	18	96	16.7	170/90
51	35	182	33	0	+	150/70	-	0.7	41	48	33	16	110/60
39	10	641	53	1.2	+	150/95	+	6.4	35	46	761	13.4	170/110
32	27.9	351	45	Average		153/91		4.0	3.5	28.1	177	14.9	163/96
14	22.4	181	11	9 D		18/15		3.1	14.5	16.8	107	6.3	240/16
3	4.5	36	2	5.8 M		3.5/3.0		0.6	2.9	3.4	31	1.4	4.8/3.1

5 mg/kg b.wt. and thereafter 1-3 mg/kg/day dependent on WBC. Prednisone therapy was initiated with a high initial dosage. The first 11 patients (T 75-T 137) were initially given 250 mg/day the remaining patients (T 138-T 185) 150 mg/day. Dosage was reduced during the course of 3-4 week to 30-40 mg/day. In five transplantations (T 135, 140, 153 +b and 156) severe acute rejection episodes were seen and grafts were destroyed in T 135 and T 153 a+b. The postoperative course was complicated by severe infections with lethal termination in T 125 and T 138. T 161 became anuric during the second posttransplant day and graft anuria lasted for 16 days. In 16 patients the postoperative course was without complications.

METHODS

14 recipients simultaneous male 125 I-iothalamate creatinine PAH and 125 I-hippuran clearances were determined 1-2 hours postoperatively (4-17). All patients received parenteral fluids (0.9% NaCl 100-250 ml/h). At the time of the first determinations all patients had arterial catheters through which graft urine was collected. Thereafter clearance studies were performed within 2-6 days and again 7-20 days post transplant. An attempt was made to relate graft function to donor's age, warm, cold and total ischemia times. Changes in functional pattern were followed during the first 20 posttransplant days.

For calculation of *p*-values Spearman test was used together with Wilcoxon's rank sum test.

RESULTS

As shown in Table 1 initial graft function was independent of donor's age. In 13 recipients with kidneys from donors less than 35 years of age the mean 125 I-iothalamate clearance was 24.9 ml/min which is not different from the mean 125 I-iothalamate clearance for the whole study group. Further more there was no correlation between donor's age and level of initial graft function (Table 1).

Warm ischemia time i.e. length of time from cessation of circulation to start of perfusion of the isolated kidney varied from 0 to 84 min. The cold ischemia time i.e. length of time from start of perfusion to reestablishment of renal circulation varied from 102 to 787 min. In 17 recipients with a warm ischemia time less than 30 min the mean initial 125 I-iothalamate clearance was 23.4 ml/min a figure similar to that of the total study material. Correlation between initial graft function and length of warm ischemia time could not be demonstrated during the interval of 0-84 min. Neither could length of cold ischemia time be correlated to initial graft function.

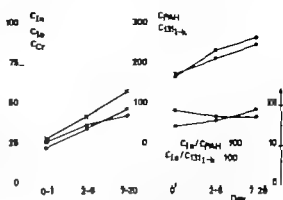


Fig. 1 Mean GFR (ml/min), ERPF (ml/min) and FF values obtained during the first 20 postoperative days in recipient of cadaveric renal transplants.

In 17 grafts with warm ischemia time less than 30 min the cold ischemia time was longer than that in the remaining 8 grafts with a warm ischemia time longer than 30 min. The mean cold ischemia time was 395 and 760 min respectively. This difference is however not significant. The sum of the warm and cold ischemia times did not correlate to initial graft function.

Graft function expressed as mean 125 I-iothalamate and 125 I-hippuran clearances increased during the course of the first 6 posttransplant days by 50 and 35% respectively and during the following 14 days by 35 and 15% (Tables I, II, III and Fig. 1). PAH and 125 I-hippuran clearances were identical at the first measurement whereas 125 I-hippuran clearance during the following two periods of measurement was somewhat lower than PAH clearance. All measurements revealed good correlation between PAH and 125 I-hippuran clearances ($p < 0.001$). Serum albumin at three measurements was 3.34, 3.28 and 3.63 g/100 ml respectively. Proteinuria varied markedly irrespective of donor's age and ischemia time. The mean BP level varied only slightly during the study period. The average diastolic BP increased 7 mmHg in relation to values before transplantation. Creatinine clearance values at the time of the three measurements were 20-35% higher than 125 I-iothalamate clearance values but good correlation was found ($p < 0.001$).

The filtration fraction (FF) at the time of the first measurement immediately after transplantation was significantly reduced (13/14.9%) being found in the following measurements FF 0-

Table II Kidney function on days 2-6

Abbreviations as in Table I

T no	Proteinuria (g/24 h)	C_{cr} (ml/min)	C_{cr} (ml/ml)	C_{125I} (ml/ml)	FF C_{cr}/C_{125I}	BP
83	0.5	26	26	159	16.4	155/95
96	0.5	37	49	69	13.8	190/100
101	2.4	29	40	184	15.8	140/90
102	2.6	54	77	448	14.1	190/110
105	1.3	64	84	506	12.6	125/80
111	1.6	28	33	194	14.4	150/90
112	3.2	16	70	103	15.5	160/135
118	1.0	64	91	356	18.0	170/90
125	2.7	8	12	58	13.8	190/110
135	3.7	1	77	128	16.4	160/80
137	1.9	60	67	306	19.6	140/90
138	3.4	38	43	164	13.4	140/90
140	0.7	1	16	179	9.3	160/90
141	1.6	15	19	89	16.9	10/120
143	0.7	64	71	370	20.0	180/120
149	1.0	43	44	222	19.4	155/105
153a	9	26	37	159	16.4	135/90
153b	1.7	17	22	129	11.2	140/80
154	0.5	15	20	84	17.9	180/110
156	0.7	28	38	264	10.6	190/100
172	3.1	16	24	114	14.0	170/90
178	0.9	66	67	310	1.3	130/70
185	1.4	63	71	287	22.0	170/90
Average	1.7	35.4	43.4	17	16.2	16.7/97
S.D.	1.0	20.1	4.1	119	3.6	23/15
S.E.M.	0.4	4	5.0	45	0.8	4.8/3.4

Table III Kidney function on days 7-20

Abbreviations as in Table I

T no	Proteinuria (g/24 h)	C_{cr} (ml/min)	C_{cr} (ml/min)	C_{125I} (ml/min)	FF C_{cr}/C_{125I}	BP
75	0.3	97	92	370	4.9	180/115
83	0.0	46	46	776	16.7	150/95
96	0.0	39	54	288	13.5	160/90
101	0.5	41	57	237	17.3	170/110
102	1.2	77	76	368	20.9	180/100
105	0.5	51	69	380	13.4	160/100
111	0.6	49	69	260	18.8	160/80
112	4.5	26	41	119	21.8	185/120
118	0.8	82	96	374	21.9	165/100
125	1.2	14	21	76	18.4	180/130
137	1.8	63	66	394	16.0	140/80
140	0.0	22	28	146	15.1	140/90
141	1.0	22	27	114	19.3	180/115
143	0.0	63	87	309	20.4	175/110
154	0.0	40	65	233	17.2	150/75
156	0.0	53	66	315	16.8	180/80
172	0.2	32	50	159	20.1	170/90
178	0.0	65	51	277	23.9	160/85
185	0.0	65	90	267	24.3	170/105
Average	0.7	49.6	60.3	261	19.0	166/98
S.D.	1.1	21.6	22.3	99	3.4	14/15
S.E.M.	0.2	5.0	5.1	23	0.8	3.2/3.3

creased showing significantly different values from first to third measurement ($p < 0.001$). In the five cases in which severe rejection was seen 5–8 days after transplantation, the FF during days –6 was less than the mean for the whole group namely 13.2, compared to 16.2%.

DISCUSSION

Twenty four patients who had received 25 cadaveric kidneys were studied. All demonstrated an initial graft function which expressed as creatinine clearance was greater than 8 ml/min. An evaluation was made of graft function in relation to the age of the donor and the length of the ischemia time. In addition the functional pattern was described.

In recipients of cadaveric transplants it is difficult to evaluate which factors are of greatest significance for graft function. The donor's condition before death varies and is often unknown and the length of the warm ischemia time is difficult or impossible to determine precisely.

We found no relationship between donor's age and initial graft function. Neither was there any correlation between graft function and warm or cold ischemia times. It has previously been shown (3) that, if the donor has not been hypotensive (BP < 80 mmHg for > 1 hour) and the warm ischemia time is not longer than 130 min, urine production will be seen in all transplants within the first 4 postoperative days. It has also been shown that the length of the warm ischemia time if < 40 min (15) does not influence graft survival.

All donors had been hospitalized for at least 4 hours and BP and urinary production values were known for 24 donors. The donors' conditions before death and the lengths of warm ischemia times were also relatively well characterized in the present study.

Results obtained in recipients of cadaveric transplants were compared with a group of recipients receiving kidneys from living donors (7). The ages of cadaveric kidney donors were significantly less than those of living donors. Ischemia times in transplants from living donors were short and well defined (7). At all measurements of graft function in cadaveric transplants as expressed by ^{125}I -iothalamate and ^{125}I -hippuran clearances significantly lower values were found than in recipients of kidneys from living donors: the glomerular

filtration rate (GFR) increased during the period studied whereas the effective renal plasma flow (ERPF) remained almost constant. Also in the present study the ERPF increased but less than the GFR. The prolonged discontinuance of renal blood supply thus caused damage both to glomerular and tubular function, which did not disappear during the first 20 days after transplantation.

During the study period the graft function increased independently of the donor's age and the warm and cold ischemia times. This is in agreement with the observation that compensatory hypertrophy is independent of age (12). In contrast Ogden (11) found after unilateral nephrectomy in living donors an inverse correlation between donor's age and compensatory hypertrophy of the remaining kidney. A similar inverse correlation between donor's age and compensatory hypertrophy in recipients could not be shown.

The FF $\text{ml}/100\text{ ml}/\text{h}$ was significantly lower than normal values (13) in all measurements but was identical to the values obtained in recipients of transplants from living donors (7). FF at the time of the first measurement was significantly lower than that at the time of the third measurement. This means that the ERPF reached maximal values more rapidly than the GFR. Changes cannot be due to variations in BP or serum albumin concentrations since these values were constant during the whole study period. In five patients very low FF values were seen in connection with acute rejection episodes (Table II). Thus changes in functional pattern were demonstrated in connection with ischemia and rejection, a reduction in FF being found (Tables I and II). This is also manifest in phase II of the isotope renogram where the speed of accumulation in these situations is higher than the speed of elimination (6, 8).

This study of initially functioning cadaveric kidneys, for which the ischemia times are long and variable, revealed a characteristic change in GFR and ERPF. Initial graft function did not correlate in donor's age or variations in warm and cold ischemia times which were maximally 84 and 787 min respectively. The mean function during the study period was however significantly lower than the function in recipients of kidneys from closely related donors for which the ischemia times were much shorter. Twenty days post-transplant the mean ^{125}I -iothalamate clearances were 74% of the function in living kidney donor

grafts and ¹²⁵I-iothipuran clearances were 77%. FF values did not vary significantly in the two study groups

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IMMUNOPATHOLOGICAL CHANGES DURING IMMUNOSUPPRESSIVE TREATMENT OF GLOMERULONEPHRITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract. Fluorescent microscopy examination of up to 4 consecutive renal biopsies has been performed during combined therapy with azathioprine and prednisone in each of 7 patients with SLE and glomerulonephritis. The changes in the immunopathological picture after treatment showed good correlation to the changes in renal function. In 2 cases progression of immunofluorescent changes corresponded well to progression of renal insufficiency. The pattern of immunofluorescence microscopy and renal function was unchanged in other cases. In 2 cases an improvement in the immunopathological findings corresponded with an improvement in renal function. One patient died shortly after the commencement of the treatment. Progression was seen particularly in the cases in whom the initial immunofluorescence pattern was very lumpy. In contrast the immunosuppressive therapy had the best effect in the cases with focal or diffuse granular immunofluorescence patterns.

The development of lupus nephritis is probably related to an immunological disorder and the disease represents a form of immune complex induced glomerulonephritis (6, 11, 16, 17, 18). Immunoglobulin and complement are deposited in the renal glomeruli. In severe cases similar deposits are found also in the extraglomerular tissue. The immunoglobulins and complement are usually located in identical sites and indicate immune complexes (6, 16). The pattern and severity of immune complex deposition in immune complex nephritis is correlated to the degree of nephritis (8). Diffuse lumpy or diffuse granular deposits along the glomerular capillaries of all or most lobules are found in severe cases, while diffuse mesangial or focal immune complexes are seen in mild disease (11, 17). Focal granular deposits are also seen in the early stages of lupus nephritis (1).

Cytotoxic drugs with immunosuppressive effect such as purine antagonist azathioprine (Imurel®) and the alkylating agent cyclophosphamide (Sandozan) have been tried in SLE-nephritis. However the effect has not been correlated extensively to renal function and immunopathological changes. This report describes the changes in the renal immunopathological picture during combined Imurel and prednisone treatment in 7 patients with lupus nephritis.

MATERIAL AND METHODS

Patients

The 7 patients, 4 females and 3 males, all fulfilled the ARA criteria for SLE. All had positive tests for anti-nuclear antibodies detected by the LE factor test or ANF test. The renal function was determined by usual and PAH clearances, examined a few days prior to the treatment and repeated after 3-27 months, usually at intervals of about 6 months as described previously (4).

Biopsies

Consecutive needle biopsies were drawn as reported earlier (3). A biopsy was always taken before combined therapy was initiated and up to 3 sequential biopsies were examined during the treatment period. They were obtained simultaneously with the renal function studies. The specimen was at once embedded in Tissue-Tec OCT Compound (Anes Co.) quick-frozen in dry ice/acetone and isopentane and if not examined immediately OCT blocks were stored at -70°C. Cryostat sections were cut approximately 4 µ thick, mounted on glass slides, fixed in cold acetone for 10 min, washed and stained with fluorescein isothiocyanate or fluorescein rhodamine B-labelled antibody globulin as previously described (20). The methods used for UV microscopy and microphotography have been published elsewhere (20).

Table 1 Change in immunopathology compared with conventional histology and renal function after combined immuno suppression treatment of SLE-glomerulonephritis

II=definite improvement I=slight improvement, U=unchanged P=progression

Pat. no.	Age (y)	Sex	Treatment period (mo.)	Consecutive biopsies examined	Changes after treatment			Initial immunofluorescence pattern
					Immunofluorescence	Light microscopy	Renal function	
1	23	♂	1/4	Autopsy				Diffuse lumpy
2	34	♀	8	3	P	P	P	Diffuse lumpy
3	38	♀	25	4	P	I (focal)	I	Diffuse granular
4	1	♀	19	3	P	P	II	Diffuse lumpy
5	5	♂	77	4	II	U (focal)	I	Diffuse granular
6	41	♀	—	3	P	P (focal)	U	Focal granular
7	45	♂	3		U	Unsuccessful		Diffuse granular

*Very fine granules**Antisera*

Antisera to the immunoglobulin classes IgG, IgA and IgM, the complement component C1q and C3, fibrinogen and albumin were made by rabbit immunization using isolated protein in complete Freund's adjuvant (19).

Antibody γ -globulin was isolated, labelled by fluorochrome (FITC or rhodamine) absorbed and tested for specificity with sensitive hemagglutination or fluorescent labelling techniques as reported in previous publications (20).

Human sera

Sera were collected from patients simultaneously with performance of biopsies and stored at -20°C . For complement examination fresh sera were kept frozen at -70°C .

Nuclear antibodies and nuclear antigens

Indirect LE test on serum, the method of Larsen and Boyum (13) was used. Indirect ANF test was carried out with mouse liver cell nuclei antigen (20). Antibodies to native and heat-denatured DNA were detected by immunodiffusion as described by Koffler et al. (11). Nuclear antigens in tissue were traced by using fluorescein-labelled isolated antibody IgG from a patient with precipitating anti-DNA antibodies.

Complement

The complement component C3 was quantitated in serum by Mancini technique with precipitating rabbit antibodies (15). C3 in a normal serum pool was used as standard. For titration of the complement component C1q, 0.1 M Na₂ EDTA to 0.025 M Na barbital buffer was added to a part of the serum in a dilution of 1:10. The serum titre of C1q was obtained by latex immunoglobulin flocculation (18).

RESULTS

Immunological findings prior to combined therapy

Prior to the combined treatment all kidneys contained diffuse lumpy or granular deposits or in

one case focal granular deposits of immunoglobulin and complement in the glomeruli. The initial immunofluorescent patterns of immunoglobulins and complement in the glomeruli are shown in Table 1. The granular deposits in cases 3 and 5 were seen as regular beads on a string along the glomerular capillary walls (Fig. 1). In patient 3 the granules were smaller than usual and the fluorescence relatively weak. In case 7 the granules varied in size and shape and were confluent in smaller areas. However the pattern was recorded as lumpy only when granules flowed together into larger homogenous masses. In the cases with focal alterations several glomeruli were found without fluorescence and the affected glomeruli had most often lobular deposits of immunoglobulin and complement.

In addition to the major deposits of immunoglobulins and complement factors in the glomeruli, immune complexes were also detected in the walls of extraglomerular vessels in two patients (nos. 1 and 4) in the tubulus cells, tubular basement membranes and throughout the interstitial connective tissue. Albumin was detected only in collections of small droplets intracellularly in the proximal tubulus (Fig. 1). Fibrogen most often as lumpy or granular deposits was seen in the glomeruli and in the connective tissue only in the most severe cases. In a few instances isolated deposits of complement C3 without immunoglobulin were detected particularly in Bowman's capsule and along the tubular basement membrane (Fig. 3). The distribution of the initial localization of proteins in the different structures of the renal tissue is shown in Table 2.



Fig. 1 Kidney biopsy from patient 5. Glomerular deposits seen as 'beads on string' along the glomerular capillary wall (stained with FITC-conjugated anti-C3). Similar staining was obtained with anti-IgG.



Fig. 2 Kidney biopsy from patient 7. Collections of small droplets of albumin intracellularly in the proximal tubuli (stained with FITC-labelled anti-albumin).

In 6 cases the immune complexes contained IgG, IgM and C3. In one of them (no. 4) immune complexes with IgA appeared later during therapy and in 3 (nos. 2, 5 and 7) only traces of IgM were present. In one case (no. 6) there was a strong immunofluorescence with anti-C3 while only traces of IgG and no IgM were present. In patient 4 the glomeruli also contained large globular bodies with nuclear antigens detected by FITC-labelled anti-DNA. In addition to immunoglobulins and complements C3 and C1q. These probably represented hematoxylin bodies.

Immunological findings after combined therapy

In Table I the degree of immune complex deposition in the glomeruli after treatment is compared to changes in renal function. Improvement or progression was determined the following way:

Improvement was recorded when there was a decrease in the amount of deposits or in intensity of immunofluorescence. A change from diffuse to focal distribution of immune complexes in glomeruli was also taken as an indication of improvement (Table III). A further sign of improvement immunologically was change in the immunoglobulin class of immune complexes from IgG to IgM (Table III). Such an alteration was not absolute but a relative change could clearly be observed in some of the cases. A progression of the condition was measured by an increase in the amount of deposition, a more diffuse distribution, and a change towards more IgG in the immune complexes.

When these criteria were utilized to measure improvement or progression, the immunopathological findings corresponded well to the changes in renal function (Table I). During the course



Fig 3 Kidney biopsy from patient 4. Isolated deposits of complement factor C3 & bound immunoglobulins along the tubular basement membrane (stained with FITC-labeled anti-C3).

a clear improvement was detected in cases (nos. 3 and 5) by a reduction of the immune complexes (Fig 4). This was most marked in patient 5 who was followed for more than 4 years with 4 con-

secutive biopsies, with a steady decrease in immune complex deposition and a change in more IgM deposits. The same trend was pronounced but less marked in patient 3. In both these patients a definite clinical improvement was observed, most marked in patient 5 but also definite in patient 3. The tendency to improvement was also observed in light microscopy of consecutive biopsies from these 2 patients. Table 1 also shows that in both these cases the light microscopy changes were described as focal while the immunological appearance was that of diffusely localized immune complexes.

A slight progression was observed in cases (nos. 3 and 4). They had heavy lumpy deposits in all glomeruli prior to the combined treatment (Fig 5). There was clearly a progression in the immunoglobulin depositions and a change to heavy lumpy IgG deposits with less IgM (Table IV). In both these cases the progression was clearly associated with a progression in the renal function and in light microscopic changes (Table 1). In one patient, however, a partial improvement occurred later (Table IV).

In cases (nos. 6 and 7) the immunopathological picture was almost unchanged. One of them was followed only for a short period while the other with focal granular deposits was followed for 11 months. In both these patients also the renal function was almost unaltered.

Relationship to other immunological findings

All the 7 patients had positive tests for antinuclear antibodies at the initial stages of the disease. In patient 4 there was a marked intensification of the immunopathological findings at one stage during the study. After 9 months of therapy one biopsy showed a large amount of immune complexes

Table 1 Protein deposition before treatment in renal tissue from 7 cases with SLE glomerulonephritis

Protein	Glomeruli	Bowman capsule	Proximal tubuli	Juxta-glomerular vessels	Interstitial tissue
IgG	7*	4	4	0	0
IgA	1	0	0	1	0
IgM	6*	1	4	3	1
C3	7	4	3	0	0
Albumin	0	0	5	0	0
Fibrinogen	3	0	1	1	0

*One had only traces.
Three had only traces.

Table III Glomerular deposits observed by immunofluorescent microscopy of renal biopsies from patient 5

Trace amounts within parentheses

Combined therapy (mo.)	Immunofluorescence pattern	Glomerular deposits		
		Quality	Intensity	Quantity
0	Diffuse granular	IgG/IgM/C3	+++	+++
6	Diffuse granular	IgG/IgM/C3	++	++
13	Focal granular	IgG/IgM/C3	++	+
27	Focal granular	(IgG)/IgM/C3	++	+

deposited in the interstitial connective tissue in vessel walls and in the proximal tubulus. A renewed biopsy 10 months later showed a decline in these extraglomerular complexes. This improvement was followed by regress of the cellular infiltration. The transitory exacerbation in this patient corresponded also to a drop in the serum complement and a rise in the serum antibody titres to native and heat-denatured DNA, ANF and LE factors were present in high titres. However, after 19 months of combined therapy ANF and LE factors were detected in significantly lower titres than before treatment and anti-DNA antibodies could not be demonstrated. The serum complement was normalized. Heavy lumpy deposits were however still present in all glomeruli but the intensity of fluorescence was somewhat weaker (Table IV). Also the renal function of this patient stopped to progress after this long treatment period.

In another patient (no. 5), who was followed for 77 months the steady improvement in immunofluorescence findings in kidney biopsies together with an improvement in renal function was also seen as a drop in serum antibody to native and heat-denatured DNA. Furthermore the complement values were normalized.

Patient 1 was examined only by an autopsy specimen after 1 week of combined therapy. He had progressive nephritis and died of renal failure shortly after the first symptoms. On the day prior to death the serum complement levels were extremely low. The serum anti-DNA antibodies were initially very high-titred but decreased simultaneously with the serum complement probably due to their blocking in immune complexes. This corresponded to the immunofluorescent findings in the renal tissue that both glomeruli and the total cortex tissue were crisscrossed with immune complex IgG and C3 aggregates.

DISCUSSION

Treatment with cytotoxic drugs such as purine antagonists and alkylating agents in combination with prednisone has been shown to improve the clinical course of lupus nephritis (4, 5, 9). However, no definite correspondence between clinical improvement measured by renal function studies, and immunosuppressive effect, measured by immunological methods, has been recorded. For this reason it has been suggested that these drugs act on non-immunological mediators (7). In the present

Table IV Deposits observed by immunofluorescent microscopy of renal biopsies from patient 4

Trace amounts within parentheses

Combined therapy (mo.)	Pattern	Glomerular deposits			Extraglomerular deposits (tubular/interstitial)		
		Quality	Intensity	Quantity	Quality	Intensity	Quantity
0	Diffuse lumpy	IgG/IgM/C3	+++	++	IgG/C3	+	(+)
9	Diffuse lumpy and granular	IgG/IgM/IgA/C3	++++	+++	IgG/IgM/C3	++++	++
111	Diffuse lumpy	IgG/IgM/IgA/C3	++	++	IgG/IgM/C3	++	++

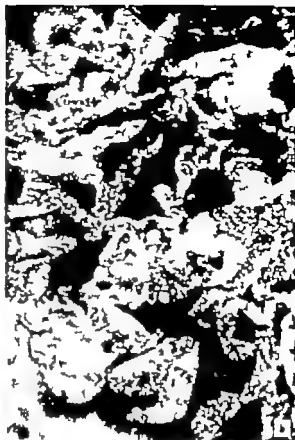




Fig 5 Sections from the kidney biopsies of patient 4. Before treatment there were diffuse lumpy deposits as detected by FITC-conjugated anti-C3 (a) and anti-IgG (b). During therapy the lumpy deposits remained and partly progressed (c) 9 months after initiation of the therapy



study however during the treatment with combined immunosuppressive drugs there was a definite decline in the glomerular immune complex deposition in two cases. This effect clearly corresponded with an improvement in the renal function and the clinical course. During the therapy it was also shown that the pattern of immunoglobulin and complement deposition could change from diffuse granular to focal. Furthermore, in these cases there was a tendency to shift from IgG to IgM deposition of glomerular immune complexes. This is in accordance with other findings of changes from IgG to IgM, when a positive effect of immunosuppression is observed (2, 14, 22).

Fig 4 Kidney biopsy from patient 5 had initially extensive diffuse granular deposits. Staining both with FITC labelled anti-C3 and anti-IgG (a and b). After 77 months of therapy the deposit was changed to focal granular staining with anti-IgG and anti-C3. Fig. shows some staining with anti-IgG, while another glomerulus is almost unstained (c).



The two cases in whom the best improvement was seen were not among the most severely affected. In contrast the two patients with the most diffusely localized lumpy deposits (nos 7 and 8) did not show any improvement but a further progression of the immunopathological findings also related to a progression of the renal failure. Even in these cases some effect on the active deposition of immune complexes could not be ruled out. The absent clinical effect might be due to irreversible tissue damage. However in patient 4 a small improvement was initiated after long treatment indicating that some improvement could be achieved in such cases. Our findings also supported the assumption that patients with diffuse lumpy deposits or irregular large granules had the most severe nephritis with pronounced renal failure. The patients with small and regular non-confluent granules were clearly those who had as a rule better renal function and responded most satisfactorily to the combined treatment. It thus appeared that in these stages the immune complex depositions were more easily reversible in contrast to the late and terminal stages where functional tests and light microscopic changes deteriorated unrelated to the immunopathological picture. This can also explain why the renal function in patient 4 progressed while at one stage there was a slight regression of immune complexes.

The immunopathological findings and the renal function studies were also compared with the findings of light microscopy (Øystese et al. in prep.). This showed mostly a corresponding improvement or progression to that observed by immunofluorescent and renal function studies. However in one case there was an unchanged light microscopy picture while the immunofluorescence and renal function studies showed a marked improvement (no 5) whereas in another case (no 6) there was a slight progression of the light microscopy findings.

In lupus nephritis immunofluorescent examination of renal biopsies can give information about the cases in which a favourable effect of immunosuppressive therapy can be expected. It is therefore possible that this method can be used for selection of patients for therapy. In addition it is useful to follow the therapeutic results particularly in the early disease stages. If the treatment can be started before the deposits have become irregular confluent or lumpy the result is assumed to be

better. In addition to immunofluorescent examination of biopsies the effect of the treatment should also be followed by quantitation of anti-DNA antibodies, complement components, immune complexes and cryoglobulins in the patient's serum (11).

The findings indicate clearly the importance of immunofluorescence examination of kidney biopsies at an early stage of lupus nephritis. If combined therapy is initiated before irreversible changes have occurred a different course of the disease with a longer survival could be expected.

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BLOOD PRESSURE DEVELOPMENT AND CHARACTERISTICS OF SUBJECTS WITH MODERATE BLOOD PRESSURE ELEVATION

A 10-year Follow-up Study in a Random Population Sample

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Abstract. In an ongoing multifactor primary preventive trial in Göteborg, Sweden, one third of all men born in 1918-19 were invited to screening examination including history-taking and blood pressure (BP) and heart rate (HR) measurement. The screening examination was attended by 1427 men (76%). Those who were considered to have borderline BP (i.e. SBP 160-175 or DBP 95-115 mmHg), were reexamined two years later regarding BP and HR. Of these 493 subjects 12% were considered hypertensives, i.e. they had SBP > 175 or DBP > 115 mmHg or had been put on antihypertensive treatment. If the majority of new cases of hypertension in Sweden come from the borderline group, the incidence of new hypertension in this age (52 y) is about 2%. At the reexamination 39% of the former borderline hypertensives had normal BP. A substantial part of those with borderline BP thus had normalized their BP within two years. Those who developed hypertension were characterized by higher SBP and HR already at the initial screening examination. They also tended to have lower physical activity at work and during leisure and higher stress experience. These differences were, however, not statistically significant.

enced by environmental factors. The primary disturbance affects the autonomic nervous control. Secondary influences on both the suprarenal renal fluid control and central nervous systems are probably of importance in the later stages of the process.

Today there is fairly general agreement on the necessity of treating cases with definitely elevated BP at least if some cardiovascular complications are found (19). Since the hypertensives usually do not appear at the physician's office because of symptoms, the discovery of the disease has to rely upon screening of population groups at risk. When such a screening is performed a group with BP in the borderline zone between normotension and definite hypertension is found. It is not known how often BP measurements have to be repeated in the borderline group if an optimal number of cases is to be detected, i.e. the incidence of definite hypertension within a group of borderline hypertensives is not known. Nor do we know the difference in characteristics between those borderline hypertensives who develop definite hypertension and those who do not.

The present study is a part of a multifactor primary preventive trial in which hypertension is treated—a randomly selected population sample of Swedish men. The aims were to study the BP development during two years in a randomized male population sample with moderate BP elevation in order to find out: 1) The incidence of new cases of definite hypertension. 2) Whether there were any special characteristics seen in those cases with moderate hypertension who developed definite hypertension during two years.

It has repeatedly been shown that hypertension is a condition which cannot be well defined by certain blood pressure (BP) limits. The BP distribution in the general population is unimodal and tailed to the right (1-20). The relatively few cases with secondary hypertension and extensively elevated BP will generally not influence this distribution to any substantial degree. The unimodal BP distribution fits the hypothesis that the majority of cases with high BP have essential hypertension, i.e. the cause is not a single organ damage. Most findings in man, supported by the findings in hypertensive rats (8, 10) are in accordance with a liability to hypertension carried by several genes and influ-

STUDY POPULATION AND METHODS

The subjects for this study of moderate BP elevation as will be defined later were recruited from a multi-factor primary preventive trial which started in 1970 (20-1). The intervention group consisted of a random third of all men aged 47-55 years living in Göteborg, Sweden. The subject were born in 1915-3. The two thirds who were not examined constituted the control group for the preventive trial. In this report data from men born in 1918-19, mean age 57.3 years and belonging to the intervention group are analysed.

The screening examination began in 1970 with men born in 1915 and ended in 1973 with those born in 1930. The subjects received postal questionnaire concerning heredity for myocardial infarction and stroke, their own chest symptoms, some earlier diseases, present physical activity during work and leisure and smoking habits. Those who answered the questionnaire (84%) were invited to screening examination which included, among other things, measurement of height, weight, BP, plasma cholesterol and heart rate (HR) by ECG. 76% attended the screening examination.

Subjects with systolic BP (SBP) above 175 mmHg or diastolic BP (DBP) above 115 mmHg were referred to a second BP measurement within two weeks. The criteria for further BP determinations and/or diagnostic examination were given in previous paper (20). Subjects with screening SBP 160-175 or DBP 95-115 mmHg were reexamined after two years and constitute the material

for this study. Those with SBP<160 and DBP<95 mmHg were considered as normotensives and had no further BP control until the ordinary work-up in the preventive trial four years after the initial screening.

Of the 1427 men who attended the screening examination 628 had SBP<160 and DBP<95, while 265 had SBP>175 or DBP>115 mmHg. Of those who had SBP 160-175 or DBP 95-115 mmHg 493 participated in the reexamination whereas 41 did not.

According to the BP reading at the reexamination two years after the initial screening the subjects were divided into three groups: (A) Subjects with SBP>175 or DBP>115 mmHg ($n=41$) or subjects who had been considered hypertensives during these two years and had received antihypertensive treatment ($n=17$). (B) Subjects with SBP 160-175 or DBP 95-115 mmHg ($n=44$). (C) Subjects with SBP<160 and DBP<95 mmHg ($n=191$).

The BP both at the initial screening and at the re-screening, was measured in the afternoon in the right arm with the subject in the sitting position after about 5 min rest. A 12.5 cm wide and 26 cm long arm cuff was connected to a mercury manometer. DBP phase 5, when the Korotkoff sound disappeared, was used. Pressures were read to the nearest mmHg. At the initial screening the BPs were measured by one of several physicians at the reexamination by one nurse. HR was calculated from the ECG. Body height and weight were measured according to Rose and Blackburn (13).

Physical activity at work and during leisure was

Table 1 Classification of physical activity in the postal questionnaire

Activity during work	Score	Activity during leisure	Score
Passive	1	1 active	1
You work mainly sedentary and you do walk about much during working hours. Examples of work of this type are desk work, watchmaking and assembly of light components		You spend most of your time reading, watching TV, at the cinema or are engaged in other sedentary activities during leisure hours	
Relatively active		Some physical activity	2
You walk about quite a lot in your work but do not carry or lift heavy objects. Examples of occupations of this type are mobile office work, light industrial work, supervisory work, instructional work of type involving a lot of walking about and work as a shop assistant		You walk, cycle or are active in some other way at least 4 hours a week. This includes walking or cycling to and from work as well as Sunday walks, normal gardening, fishing, table tennis or bowling	
Active + moderate	3	Regular physical activity	3
You walk about and lift objects quite a lot or walk up stairs or slopes a lot. Examples are postmen, people working in heavy industry, carpenters, pipelayers		You engage in running, swimming, tennis, badminton, gymnastics or other sports, heavy gardening and the like. Note that this means at least 3 hours a week on an average	
Heavy manual labour	4	Regular physical coaching	4
You have a heavy manual job, lift heavy objects and exert yourself physically. Examples are forestry workers, agricultural workers, fishermen using heavy equipment and boulders, labourers		You engage in hard training and compete in running, skiing, swimming, football, handball, etc. regularly and several times each week	

Table II Classification of stress experience according to the postal questionnaire

By stress is meant state of mental tenseness, irritability, nervousness, agony or insomnia due to for example trouble with your work or at home etc.

	Score
Have never experienced stress	1
Have experienced occasional periods of stress	2
Have experienced occasional periods of stress during the last 5 years	3
Several periods of stress during the last 5 years	4
Constant stress during the last year	5
Constant stress during the last 5 years	6

analysed according to four-point scale. The four categories were illustrated with drawings in the postal questionnaire, the classification is given in Table I. The scoring of activity was done according to calculation of energy expenditure (15).

Stress experience was coded according to a six-point scale with increasing number for increasing stress experience. The questions in the questionnaire are given in Table II. Scores 5 and 6, representing constant stress experience during the last one and five years, were combined in the analysis.

In this paper the term "borderline" only indicates BP at the border between normotension and hypertension and is not defined as suggested by Jha and Schork (6).

For the statistical analysis χ^2 -analysis was used for comparison of frequencies. Analysis of variance was used for testing the hypothesis that the means of different groups were the same. When this test showed significant differences between means, Scheffé method was applied to find the location of the differences. The level of significance used was $p=0.05$.

RESULTS

SBP, DBP and HR at reexamination: the earlier defined BP groups are shown in Table III. HR was highest in those who had definite hypertension and fell significantly from group A to group C ($F=21.18$).

SBP, DBP and HR at the initial screening examination in normotensives, in the borderline BP groups and in untreated and treated hypertensives are given in Table IV. Borderline hypertensives who had SBP > 175 or DBP > 115 mmHg at reexamination had significantly higher SBP ($F=22.99$) and HR ($F=3.46$) than the other two groups of borderline hypertensives already at the initial screening examination. There was however no

Table III SBP, DBP and HR for borderline hypertensives at examination two years after the initial examination

BP group at reexamination	SBP		DBP		HR	
	\bar{x}	s	\bar{x}	s	\bar{x}	s
A (n=41)	177	13	110	10	80	11
B (n=44)	153	10	101	6	77	10
C (n=191)	139	10	88	5	72	9

Only untreated subjects.

Table IV BP and HR at the initial screening examination in relation to values at reexamination

	At initial screening					
	SBP	DBP	HR			
	\bar{x}	s	\bar{x}	s	\bar{x}	s
Normotensives (n=625)	135	12	86	7	70	12
Borderline by persistiveness at reexamination after years						
A (n=493)	154	12	101	6	75	14
(n=58)	162	8	103	7	79	15
B (n=44)	155	11	102	6	74	13
C (n=191)	151	12	100	5	74	14
Definite hypertensives (n=265)	182	19	113	12	80	15
Untreated (n=169)	186	15	114	11	79	14
Treated (n=96)	175	22	111	14	81	15

difference in DBP between the three groups at the initial examination. The mean HR at the initial examination: the whole group of borderline hypertensives ($n=75$) fell between those with definite hypertension ($\bar{x}=79$) and the normotensives ($\bar{x}=70$).

The percentage of men with low physical activity at work and during leisure (score 1), constant stress experience (scores 5-6) and the mean body weight for the three borderline BP groups are shown in Table V. No significant differences were found. There was however a tendency to a higher percentage of physically active persons at work and during leisure and of stress experience among those with SBP > 175 or DBP > 115 mmHg at reexamination (group A). The weight was lowest in the group of men whose BP decreased (group C).

The frequency of subjects with myocardial in-

Table V *Low physical activity at work and during leisure (score 1) constant stress experience (scores 5-6) and body weight in borderline hypertensives grouped according to BP groups at reexamination after two years*

BP group at reexamination	Low physical activity (°)		Constant stress (%)	B wt (t) (s)	
	At work	During leisure			
A (n=58)	35	9	23	82	13
B (n=44)	27	20	16	81	10
C (n=191)	29	21	13	78	10

fracture or stroke among the parents was also analysed in the different groups but no significant relationships were found. Neither were any significant differences found concerning smoking habits.

DISCUSSION

In this type of population studies several thousands of subjects have to be screened and a large proportion of them will eventually have to be checked regularly. It has therefore within our limited resources only been possible to conduct more special studies in subsamples of the total population screened. Such studies are planned or under way.

The present study of repeated BP measurements of a group of borderline hypertensives over a two-year interval constitutes another type of investigation. It gives some information of interest as it covers a random sample of the total population of males within a limited age range of a well defined ethnic origin and living in a limited and well defined geographic area. Thus the findings could not be due to age-related factors or to special selection of cases. On the other hand there is a certain drop-out (25%). The non-participation group might contain a somewhat higher rate of hypertensives and other cardiovascular diseases.

The BP was measured only once both at the initial screening and at the reexamination after two years. It might be argued that this is not enough to characterize a subject as normotensive, "borderline" hypertensive or definitely hypertensive. On the other hand the accuracy of using a casual BP reading to characterize groups of individuals has been well established (3, 13).

The arbitrarily chosen cut-off points of SBP 175 and DBP 115 mmHg may seem somewhat high. They have been chosen as an earlier study showed a rapidly rising frequency of hypertensive organ manifestations above these limits (18). Several factors in the screening and reexamination procedure may account for the fact that the BP distributions are shifted to the right, i.e. to higher values. These factors have been discussed previously (70).

Of the 493 subjects who initially had "borderline" BP elevation 58 (11%) could be considered as definite hypertensives two years later. From earlier studies we know that the majority of subjects who develop hypertension have had somewhat high pressures already at the first examination (14, 16). Whether this is true also for the present population is not known, as we have not reexamined the total sample yet. The minimum incidence of new hypertension according to these findings is 79 of 1477 per year or about 2%. In a study of the natural history of hypertension in a Chicago company the risk of developing sustained hypertension in 25-30 years was calculated to be 25% (14). I.e. an incidence of about 1% per year. In a study on male Israeli civil service workers the five year incidence rates of hypertension were found to increase with age and were also related to place of birth for the immigrants (7).

It might seem curious that 191 subjects or 39% of those with borderline hypertension had normal BP two years later. One important explanation of this is of a purely statistical nature. The decrease may be accounted for by regression towards the mean (11). There are however also reports of continuous decrease of BP with time. Thus Stewart in a prospective study of 53 young men with mild BP elevation without organ manifestations (17) reported that 17 subjects (32%) had a continuous fall of BP over a period of 6.7 years. Another study (2) showed a much lower percentage of BP normalization in subject with "borderline" hypertension. These studies do not however reflect the situation in the total population but in selected groups of subjects.

It must be stressed that even minor differences in BP measurement, such as time of the day, time of rest before measurement etc. make comparisons between different prevalence studies of hypertension very difficult. Hitherto we have no suspicions that the true BP distribution is different

in our city than in other communities from which studies have been published. The rate of definite hypertension and "borderline" hypertension with our cut-off points is in fairly good accordance with some other studies. Thus our prevalence of "borderline" hypertension viz. 34% is in good accordance with that found by Julius and Schork in their review of borderline hypertension (6).

The method used to study physical activity and stress experience, i.e. postal questionnaire, has the disadvantage of having to rely upon the person's subjective opinion. This study was however done prospectively which reduces the risk of bias. The finding of a tendency to lower physical activity and higher stress experience among those borderline hypertensives who developed definite hypertension ought to be checked by studies in other populations and if possible by using more objective methods.

In the present study subjects with definite hypertension had a significantly higher HR than both the normotensives and those with "borderline" BP elevation. Earlier too it has been well documented that high BP is positively correlated with HR (2, 4, 6). Furthermore those borderline hypertensives who developed definite hypertension were characterized by having a higher HR and higher SBP indicating a hyperkinetic circulation. Julius et al. (5) found this to be due both to an increased sympathetic effect and to a reduced parasympathetic effect in "borderline" hypertension. Their findings are in agreement with those in established hypertension published by Korner et al. (8). Studies in hypertensive rats have also shown the importance of increased sympathetic activity for the development of hypertension (10).

It is quite possible that the disturbed cardiovascular regulation with hyperkinetic circulation is an early event in the disturbed regulation of BP in hypertension and thus characterizes subjects with a BP progression from normotension to definite hypertension. Whether this will indicate any special form of treatment suitable for this group calls for separate studies.

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THE RELATION OF PLASMA RENIN ACTIVITY TO LEFT VENTRICULAR HYPERTROPHY AND RETINOPATHY IN PATIENTS WITH ARTERIAL HYPERTENSION

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Abstract Plasma renin activity (PRA) has been determined in 77 hypertensive patients in the supine position at 8 a.m. after 48 hours sodium restriction. Mean PRA was highest in patients with renal disease, but individual values overlapped with those from the 51 patients with "essential" hypertension. Ten patients were found to have low and unresponsive PRA (≤ 0.4 ng ang. I/ml/h; primary aldosteronism was diagnosed and ruled by operation in 3 of them. After exclusion of patients with renal disease we found in the remainder 54 patients, that the presence of left ventricular hypertrophy (LVH) was associated with higher mean PRA than in patients without LVH. The relative frequency of LVH was however higher in the 10 patients with low unresponsive PRA (50%) than in patients with normal or high PRA (43.3%). LVH was most closely related to the BP level, especially the systolic BP which was significantly higher in patients with LVH than in those without. It is concluded that the level of the BP is the single most important factor for the development of LVH while renin-angiotensin are probably of no direct importance. The patients were divided into two groups according to the severity of retinal changes. The relative frequency of the most advanced retinopathy (grades II and III after Keith and Wagener) was, unlike the occurrence of LVH, found to be lower in patients with low PRA, occurring in 1 of 10 patients as opposed to 1 of 44 patients with normal or high PRA. Presence of retinopathy grade II-III in patients with normal/high PRA was associated with significantly higher mean PRA than in patients with grade 0-1 changes. Patients with retinopathy grade II-III were not older, their hypertension had not been known for longer time, but they had significantly higher systolic and diastolic BP than patients with retinopathy grade 0-1. Renin-angiotensin may play direct role for the development of retinal hypertensive arteriopathy. The close association between high mean PRA and BP in the patients with the most advanced retinopathy could mean that angiotensin increases the adverse effect of high BP on arterioles.

In most patients with essential hypertension the plasma renin concentration (3, 4) and the plasma renin activity (PRA) (6, 10) are normal. In some

patients PRA is elevated and in 20-50% it is subnormal and fails to respond to sodium restriction and upright position (6, 10). The different PRA levels in patients with essential hypertension could imply that different pathogenetic mechanisms were operating. However, even in renal hypertension the pathogenetic role of renin-angiotensin is not entirely settled (1, 7, 8, 13, 14), and its implication in other hypertensive states is in fact more questionable.

Although the relation of the renin-angiotensin system to the development or maintenance of elevated BP is a matter of discussion, renin-angiotensin might be of importance for the development of hypertension or cardiovascular complications. This assumption is supported by the fact that in patients with primary aldosteronism and low or undetectable PRA hypertensive retinopathy is mild if present at all and cardiomegaly is seen in only 40% of the patients (9). Remarkable are the observations of Brunner et al. (6) that coronary infarction and stroke did not occur in their patients with essential hypertension and low PRA in contrast to the relatively frequent occurrence in their patients with normal or high PRA.

In patients with malignant hypertension both plasma renin concentration, angiotensin II and PRA are regularly found to be elevated (4, 8, 17, 20) as is the urinary aldosterone excretion. The activation of the renin-aldosterone axis is thought to be responsible for the accelerated course of the hypertensive disease (7, 17, 20).

The intention of the present study has been to examine the relation between the level of PRA and the occurrence of left ventricular hypertrophy (LVH) and retinopathy in patients with arterial hypertension.

Plasma renin activity
($\mu\text{g ang I/ml/hour}$)



Fig. 1 Individual and mean PRA values in 72 patients with renal hypertension determined in recumbent position for the last 10 hours and after 48 hours sodium restriction ($\text{Na} < 15 \text{ mEq/24 h}$).

PATIENT MATERIAL AND METHODS

the period Jan. 1970 to March 1973 77 hypertensive in-patients at Medical Department B Åke Hospital were extensively evaluated for secondary hypertension. The patients represent a selected group as more than 60% were referred from other hospital or specialists. Only patients with diastolic BP $\geq 95 \text{ mmHg}$ on repeated measurements were included. Patients with malignant hypertension were excluded. The mean age of the patients was 43 years (range 17–67); only three patients were older than 60 years.

The mean BP for all patients (182/116) was calculated from the average of three BP readings in each patient. These were recorded with the patient recumbent on the third, fourth and fifth mornings after admission.

Forty-six patients had been treated with antihypertensives, all treatment was stopped at least 2 weeks before the study. Some patients were referred with suspicion of primary aldosteronism because hypokalemia had developed after thiazide diuretics. They were given potassium supplement, and serum sodium and potassium were within the normal range at the time of the study except in 4 patients who were still slightly hypokalemic.

One intention of the study was to compare the value of rapid sequence pyelography and isotope renography as screening procedures for renal hypertension. Both these examinations, performance of renal angiography as well as determination of peripheral vein PRA and renal vein PRA were done in the first 60 patients studied.

In the last 12 patients isotope renography alone was used as screening procedure for renal hypertension, as the method described by Norman (23) has been shown to be a reliable screening method for renal hypertension (18).

The peripheral vein PRA values compared to hypertensive complications in the present communication were measured at 8 a.m. after 48 hours sodium restriction ($\text{Na supply} < 15 \text{ mEq/day}$) with the patient recumbent for the last 10 hours. PRA (angiotensin I generation rate) was determined radioimmunochemically (27) and expressed as ng angiotensin I generated per ml plasma per hour (ng ang. I/ml/h).

In all patients with PRA below $0.5 \text{ ng ang. I/ml/h}$ and in most others we also examined the PRA response to upright position and an acute oral load with furosemide. Primary aldosteronism was diagnosed when PRA was suppressed and unresponsive together with the finding of a normal or high secretion rate of aldosterone not suppressed by sodium loading.

The degree of retinopathy was classified from 0 to IV after Keith and Wagener. All the fundi were evaluated by the same observer (H. J.) who at that time did not know the actual value for PRA. LVH as diagnosed from ECG adopting the simplified score system described by Skjaggestad and Kjerfve (25) and from cardiac X-ray. LVH was said to be present when $S_{\text{max}} + R_{\text{max}}$ in V_{1-4} were 40–49 mm either in combination with a Q-wave in V_{1-4} or in combination with radiological signs of cardiac enlargement (heart volume: women $\geq 400 \text{ mm}^2$, men $\geq 400 \text{ mm}^2$). $S_{\text{max}} + R_{\text{max}}$ in $V_{1-4} \geq 50 \text{ mm}$ were alone regarded as sufficient to diagnose LVH.

RESULTS

Among the 72 patients primary aldosteronism was diagnosed and verified by operation in 3. In 18 patients the hypertension was caused by or associated with renal parenchymal disease or renal arterial stenosis (4 were operated upon). In the remaining 51 patients no possible cause for the hypertension was found and their hypertension was classified as "essential". The mean PRA in the renal disease group ($3.44 \text{ ng ang. I/ml/h}$) was significantly higher ($p < 0.0025$) than that of the essential hypertension group ($1.85 \text{ ng ang. I/ml/h}$). The individual PRA values were however overlapping in the two groups as shown in Fig. 1. Similarly in some patients with essential hypertension low and unresponsive PRA was found as in patients with primary aldosteronism.

Table 1 PRA (ng ang. I/ml/h) mean and range) in relation to type of hypertension and to the presence or absence of LVH and the grade of retinopathy in 72 hypertensive patients

Type of hypertension	LVH absent		LVH present		p	Grade of retinopathy						p
						0-I		II-III				
		PRA		PRA			PRA		PRA			
"Essential"	51	28 1.51 (0.4-4.8)	23	2.27 (0.6-6.8)	<0.05	38	1.46 (0.2-4.8)	13	3.0 (0.6-8.7)	<0.005		
Renal disease	18	8 2.86 (0.9-5.5)	10	3.90 (1.4-5.7)	<0.30	14	3.1 (0.9-4.6)	4	4.54 (3.6-5.7)	<0.20		
Primary aldosteronism	3	2 0.4 (0.2-0.2)	1	0.3		3	0.23 (0.2-0.3)					
Total	72	38 1.72 (0.4-5.5)	34	2.69 (0.6-6.8)	<0.01	55	1.83 (0.2-5.6)	17	3.36 (0.6-8.7)	<0.005		

In Table 1 the presence of LVH and the degree of hypertensive retinopathy are related to the mean PRA in the three groups of patients. The presence of LVH in patients with essential hypertension was associated with a significantly higher ($p < 0.05$) PRA (2.27 ng ang. I/ml/h) than in patients without LVH (1.51 ng ang. I/ml/h) whereas mean PRA in patients with renal disease and LVH (3.90 ng ang. I/ml/h) was not significantly higher ($p < 0.30$) than the value in the "renal" patients without LVH (2.86 ng ang. I/ml/h). For all patients lumped together we found a highly significant difference in mean PRA ($p < 0.01$) with the lowest value in the patients without LVH.

The same trend was found for the grade of retinopathy. In the patients with more severe changes (grades II and III) the mean PRA was higher than in patients with ophthalmopathy grades 0 and I (Table 1). The mean PRA values (4.55 versus 3.1 ng ang. I/ml/h) were not significantly different in the small number of patients with renal disease unlike the respective PRA values for the patients with essential hypertension (3.0 versus 1.46 ng ang. I/ml/h) and for all the patients lumped together (3.36 versus 1.83 ng ang. I/ml/h) in whom the mean PRA values were significantly higher ($p < 0.005$) in the group with the most advanced retinopathy. LVH was present in one of the patients with Conn's syndrome; none of them had retinopathy grades II or III.

One of the patients with Conn's syndrome had also renal disease (pyelonephritis) with serum creatinine of 2.2 mg/100 ml. As she became normotensive after unilateral adrenalectomy she

had certainly not renal hypertension and is therefore not included in the renal disease group.

After exclusion of the patients with renal disease the remaining 54 patients were subdivided into a low PRA group of 10 patients (PRA ≤ 0.4 ng ang. I/ml/h, with no increase after ambulation and peroral furosemide) and a normal/high PRA group of 44 patients (PRA ≥ 0.5 ng ang. I/ml/h, increasing in the upright position) (Table 1). The low PRA group included the 3 patients with primary aldosteronism. Both the mean age, systolic and diastolic BP as well as known duration of hypertension and serum creatinine concentration were higher for the patients in the low renin group; only the difference in mean age was however statistically significant ($p < 0.05$).

Table 1 shows the lower frequency of retinopathy grades II and III in the patients with low PRA occurring in 1 of 10 patients; in the patients with normal or high PRA, retinopathy grades II or III was found in 1 of 44. In contrast to this the relative frequency of LVH was even higher among the 10 patients with low PRA in whom LVH was diagnosed in 5 as opposed to only 19 of the 44 patients with normal or high PRA.

Table 1 shows that the mean systolic BP in the low PRA group was significantly higher in patients with signs of LVH than in those without. This holds true also for the patients with normal or high PRA in whom the presence of LVH was associated not only with a higher systolic and diastolic BP but also with a significantly higher mean PRA than in patients without LVH.

Patients with LVH did not significantly differ in

Table II Occurrence of LVH and the grade of retinopathy in 34 patients with arterial hypertension classified according to low and normal/high PRA

PRA group	n	Mean values for		Age (yr)	Known duration (yr)	Serum creatinine (mg/100 ml)	PRA, mean and range (mg ang. I/ml/h)		
		BP (mmHg)					Supine	Upright	After furosemide
		Syst	Diast						
<0.4	10	189	116	50.0	5.5	1.06	0.26 (0.2-0.4)	0.26 (0.2-0.4)	0.3 (0.2-0.4)
≥0.5	44	180	115	41.4	4.83	0.97	1 (0.4-6.8)	3.2 (0.8-10.0)	
III		<0.30	<0.90	<0.05	<0.10	<0.30			

mean age, known duration of hypertension or serum creatinine concentration from patients without LVH either in the low or the normal/high PRA group.

In Table IV the same comparison is made for the grade of retinopathy. In the high/normal PRA group retinopathy grades II-III was associated not only with a higher mean PRA but also with a higher mean systolic BP than retinopathy grade 0-I. There was no significant difference in the mean age of the patients nor in the known duration of their hypertension but the serum creatinine concentration was significantly higher in those with the most advanced retinopathy. In the low PRA group only one patient had fundi changes grades II-III (grade II).

DISCUSSION

The finding of higher mean PRA in hypertensive patients with renal disease than in patients with essential hypertension is in accordance with the observations of others (17) and so is the marked overlapping in individual values for PRA in the two groups, a fact that makes the determination of PRA in peripheral venous blood of limited value in differentiation between essential and renal hypertension.

For evaluation of the PRA the conditions under which it is determined are most important. Brunner et al. (6) have emphasized that the classification of essential hypertensives in low, normal or high renin groups should be done only in relation to the patients' sodium balance. Whereas the influence of sodium restriction on the plasma concentration of renin and angiotensin II is a matter

of discussion, present information indicates that PRA increases (3, 9, 8, 10). Our own observations (unpublished) indicate that the adopting of an upright position in an individual on unrestricted sodium diet might be a more potent stimulus to renin release measured as PRA than sodium restriction in a recumbent individual.

The PRA in the present study was determined after 48 hours sodium restriction with the patients recumbent after 10 hours in bed. The stimulus of this regimen on PRA is probably of the same order or somewhat less than in ambulatory individuals on unrestricted sodium diet.

As the PRA has not been higher than 0.4 mg ang. I/ml/h in any of our patients with primary aldosteronism examined on this regimen, we have somewhat arbitrarily chosen this PRA level as the upper limit for low suppressed renin. An important criterion for inclusion in the low renin group has been that PRA should be not only low but also unresponsive. The lack of increase in PRA after occupation of upright position and administration of furosemide clearly separated the low renin hypertension group from the other patients.

Including 3 patients with Conn's syndrome and excluding patients with renal disease, 10 of the patients (18.5%) had a low and non-responsive PRA, a somewhat lower figure than that reported in larger series of hypertensive patients selected according to other criteria (6, 10). The effect on PRA of previous antihypertensive treatment was probably negligible, as all medication of the treated patients was stopped at least 14 days before the study.

We have found the mean PRA to be higher in

LVH (n)		Grade of retinopathy (n)	
Absent	Present	0-I	II-III
5	5	9	1
5	12	3	1

the patients with the most advanced retinopathy (grades II and III) and in patients with signs of LVH compared to patients with retinopathy grades 0-I and without LVH. If PRA is assumed to reflect circulating angiotensin II concentration, one may ask whether the positive relation between a high PRA and hypertensive complications is an expression of a direct pathogenetic role of angiotensin for the development of LVH and retinopathy or whether they are all common manifestations of the increased arterial pressure. In this connection the frequency of LVH and of retinopathy grades II and III in the low renin patients compared to the frequency in the normal/high PRA patients is of interest. The occurrence of LVH in 40% of the patients with low PRA

which was even higher than in the normal/high PRA group contrasted with the paucity of changes in the optic fundi of these patients as opposed to the occurrence of retinopathy grades II and III in more than one fourth (28%) of the patient with normal/high PRA. The difference in the distribution of the hypertensive complication in the two PRA groups could not be accounted for by differences in mean BP, serum creatinine concentration or in the known duration of the hypertension.

It is known that hyaline arteriosclerosis may represent changes due to ageing and is accentuated by chronic arterial hypertension (15). Age, however, could not be a factor of importance in the present patients since the mean age of the low PRA group in whom the retinopathy was less pronounced was significantly higher than that of the normal or high PRA group.

From our observation it seems improbable that the development of LVH is directly influenced by the level of PRA. In both PRA groups it was evident that the occurrence of LVH was most closely related to the BP, patients with LVH having significantly higher BP than the patients without. Although the mean PRA was also higher in the normal/high PRA patients with LVH, the findings strongly support the opinion of Pickering (4) that the level of the BP is the more important factor for the development of LVH that may be regarded as a response to increased working load.

Although the number of patients is small and the

Table III LVH in relation to PRA and some other characteristics in 34 hypertensive patients (mean value and range)

	PRA ≤ 0.4		<i>p</i>	PRA ≥ 0.5		<i>p</i>
	LVH absent (n=5)	LVH present (n=5)		LVH absent (n=23)	LVH present (n=19)	
PRA (ng ang./l/ml/h)	0.3 (0.2-0.4)	0.76 (0.2-0.4)	<0.05	1.65 (0.5-4.8)	2.68 (0.8-6.8)	<0.05
Systolic BP (mmHg)	177 (160-200)	200 (185-210)	<0.05	172 (160-195)	197 (160-210)	<0.001
Diastolic BP (mmHg)	113 (105-125)	118 (100-135)	<0.40	111 (95-130)	119 (100-130)	<0.01
Age (y)	53.6 (39-67)	46.4 (38-64)	<0.30	40.0 (18-47)	43.3 (27-58)	<0.30
Known duration of hypertension (y)	7.30 (0.5-4)	3.70 (0.5-10)	<0.30	5.6 (0.5-15)	3.18 (0.5-11)	<0.60
Serum creatinine (mg/100 ml)	1.14 (0.8-1.5)	1.0 (0.8-1.1)	<0.70	0.90 (0.6-1.2)	0.94 (0.7-1.6)	<0.40

Table IV Retinopathy in relation to PRA and other characteristics in 54 hypertensive patients (mean value and range)

	PRA ≤ 0.4		P	PRA ≥ 0.5		P
	Grade of retinopathy			Grade of retinopathy		
	0-1 (n=9)	II-III (n=1)		0-1 (n=3)	II-III (n=1)	
PRA (ng ang. L/ml/h)	0.39 (0-0.4)	0		1.68 (0.5-4.8)	3.3 (0.8-6.8)	<0.05
Systolic BP (mmHg)	186 (160-191)	190		175 (160-191)	195 (170-230)	<0.001
Diastolic BP (mmHg)	119 (100-135)	120		111 (95-130)	123 (105-135)	<0.001
Age (y)	58.4 (38-67)	64.0		40.0 (18-57)	45 (37-58)	<0.001
Known duration of hypertension (y)	5.55 (0.5-4)	5.0		6.69 (0.5-15)	3.3 (0.5-10)	<0.70
Serum creatinine (mg/100 ml)	1.1 (0.8-)	0.8		0.89 (0.6-1)	1.01 (0.7-1.6)	<0.05

limitations of funduscopy being a subjective method should not be forgotten the finding of less severe arteriolar changes in the fundi of patients with low suppressed PRA compared to the more venous changes in the patients with normal or high PRA could support a pathogenetic role of angiotensin for the development of arteriolar damage especially since there was no difference in mean or in renal function judged from the serum uric acid concentration in the two groups. Neither was there any statistically significant difference in the known mean duration of the hypertension but this of course is an uncertain measure of the real duration.

The finding in the normal/high PRA group that the higher mean PRA in the patients with the most accentuated arteriolar changes was also accompanied by a higher mean arterial BP as well as by a higher mean serum creatinine concentration does not exclude an independent or at least a synergistic role of angiotensin for the development of hypertensive arteriopathy. Irrespective of the primary cause in some patients a vicious circle might be started in which a high arterial BP renal arteriolar damage and increased secretion of renin are both cause and result.

The idea that renin or rather angiotensin may cause vascular lesions is not a new one. Clinical observations (3, 4, 6, 10, 17, 20) have shown a positive association between retinal arteriolar changes and the concentration of plasma renin and

angiotensin as well as PRA. Moreover arteriolar damage has been induced in animals after administration of angiotensin or renin-containing renal extracts (11, 15). This effect however has not been observed without a concomitant rise in BP. The vasculotoxic effect of renal extracts has been shown to be less evident after bilateral adrenalectomy (11) an indication that aldosterone might also be implicated in the pathogenesis, presumably by its effect on sodium balance. The role of sodium is supported by experiments on rats in which vascular disease was elicited by long-term injections of renin (7). The pathological changes were accentuated when the animals were pretreated with aldosterone and sodium but not when sodium was omitted from the diet.

The retinal arteriolar changes are representative of similar changes in other tissues (15). This pathological process is however quite different from the process of arterial atherosclerosis the development of which is accentuated by renal hypertension (16) the most probable explanation of the increased incidence of cerebral thrombosis and coronary infarction in patients with hypertensive disease (19). We do not know any experimental observations that might support the hypothesis that renin-angiotensin directly promotes the development of atherosclerosis. There would therefore be no reason to expect as reported by Brunner et al. (6) that the incidence of coronary infarction and cerebral thrombosis would be lower

in patients with low PRA than in other patients with comparable degree of hypertension. In other studies (1-22-26) the observations of Brunner et al have not been confirmed. Both for this reason and from the fact that the development of LVH seems relatively independent of the level of PRA, it would not be wise, as suggested by Laragh's group (6-20), to delay the treatment of high BP in patients with low PRA. Laragh's (20) suggestion that antihypertensives which also reduce PRA, ought to be preferred to agents which stimulate renin release seems more justifiable since the available data suggest that a high plasma level of angiotensin may accentuate hypertensive arteriolar damage.

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CHANGES IN HEPATIC CIRCULATION AT REST DURING AND AFTER EXERCISE IN YOUNG MALES WITH INFECTIOUS HEPATITIS COMPARED WITH CONTROLS

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Abstract Hepatic encephalopathy has been performed in 10 young males with infectious hepatitis and 13 healthy male volunteers. Hepatic blood flow (rest in the supine position, estimated with indocyanine green dye) in constant infusion was significantly higher in the patients than in the control (1.1 and 1.44 l/min respectively). During supine leg exercise with heart rates about 170 beats/min the absolute reduction of total hepatic blood flow was similar in patients and control. The arterio-hepatic venous oxygen difference during exercise as compared with that at rest was doubled in the patients, but tripled in the control. The splanchnic oxygen uptake at rest was significantly higher in the patients than in the controls and did not change significantly during exercise. At rest the patients had significantly greater difference between wedged and free hepatic venous pressure than the controls possibly due to swelling of the hepatocytes. The hyperkinetic hepatic circulation might have been caused by cellular damage and blood flow impairment with anoxia and metabolically evoked vasodilatation and also by an increased regeneration of liver cells, as splanchnic oxygen uptake was elevated. Immunologic factors may also have contributed. No negative effects of the exercise were noted in 8 patients with serum bilirubin values below 4 mg/100 ml, whereas relapse and slightly delayed recovery were noted in 2 patients with serum bilirubin values of 9.6 and 5.8 mg/100 ml, respectively.

Only a limited number of studies on the hepatic circulation in patients with acute viral hepatitis have been published so far and measurements have been limited to the resting state (6, 9, 19, 23, 4, 77). The hepatic extraction of colloid particles such as radioactive gold or chromic phosphate was found to be normal (77) or decreased (6, 19) interpreted as a normal or decreased hepatic blood flow. By intrasplenic injections of radioactive iodinated serum albumin and

external or blood counting in 8 patients it was concluded that their hepatic blood flow was normal (4). Similarly in 14 patients Preissig et al. (1) calculated normal values for hepatic blood flow using indocyanine green (ICG) dye as indicator.

Different methods have been used to find out whether or not the portal venous pressure is increased in these patients. Preissig et al. (1) found normal values for wedged hepatic venous pressure as a measure of the hepatic sinusoidal pressure. On the other hand, increased intrasplenic pressures have been observed in about half of the patients studied (23). Even esophageal varices have been observed esophagoscopically but not radiologically in 35 of 87 patients (9). Thus, previous studies in patients with viral hepatitis at rest have shown fairly normal hepatic blood flows and signs of normal or slightly increased portal venous pressures.

When a normal subject starts to exercise the increase in activity of the sympathetic adrenergic fibers reduces the hepatic blood flow like the flow to most non-exercising parts of the body (2, 26, 33) and similarly a reduction occurs when changing from supine to standing position (3). Despite the absence of similar studies in patients with viral hepatitis they have since long been treated with bed rest during the acute phase of the disease. The bed rest has varied from very strict (1) to moderate (7, 3) and has been used to prevent a reduction of hepatic blood flow and thereby a possible delay of healing. However, strenuous exercise for 3 hours daily did not affect recovery time or relapse rate as compared with the patients

treated conventionally (25). Similar results were observed in patients with viral hepatitis who performed a moderately heavy bicycle exercise (8).

The present investigation a preliminary report of which has already been published (17) was therefore undertaken in patients with infectious hepatitis to find out whether or not their hepatic circulation was normal at rest whether the flow was reduced in the normal way during supine leg exercise and whether this exercise caused any signs of impaired liver function.

MATERIAL

After their informed consent 10 afebrile males with HBsAg-negative viral hepatitis and 13 healthy male volunteers were studied in the morning without premedication. Although being sporadic the cases were diagnosed as infectious hepatitis since clinical and laboratory findings were not consistent with infection due to Epstein-Barr virus or to cytomegalovirus; the onset of the disease was acute and the patients were HBsAg-negative as early as 5 (2-8) days after onset of jaundice. None of the 13 subjects all 20-30 years old had a previous history of heart, lung, kidney or liver disease or of drug abuse and none used alcohol in excess. In each group 3 subjects were well trained, and the others normally trained except patient 10 who was untrained. Prior to the study all patients had been allowed ambulation in the ward. The first patients were investigated just prior to their discharge. As no signs of the exercise were observed the relative work

was increased and subjects in an earlier phase of disease were studied. On the day of the study 8 patients had serum bilirubin values below 4 mg/100 ml (\bar{x} =1.99), SGOT below 140 U (\bar{x} =70.3) and SGPT below 400 U (\bar{x} =234.4), whereas the corresponding average values in the other patients were 7.7 mg/100 ml, and 515 and 855 U (Table 1). The first 6 patients and 9 controls were allowed a light breakfast 3 hours before the flow study. The remaining 4 patients and controls were studied after overnight fast.

METHODS

The subjects were studied during a resting period of about 1 hour followed by two consecutive 20 min periods of supine leg exercise on an electro-dynamically braked bicycle ergometer (10) and during 30 min after exercise. The second work load was generally twice as high as the first and chosen so as to correspond to the heaviest load the subject was expected to sustain for another 20 min period. In 7 patients and 4 controls a 15 min rest was inserted between the loads to study the wedged and free hepatic venous pressures also after the first load. Most of these subjects also participated in another study (18) to estimate the hepatic

fat content by retrograde injections of solutions of ^{99m}Tc and ^{125}I in saline into an occluded hepatic vein before the exercise. No systematic influence of these injections on the hepatic extractions of oxygen and ICG were observed when measured both before and 2-4 min after the injections in patients and controls.

Catheterization. With the subject in the supine position a right hepatic vein was percutaneously catheterized from the cubital vein under TV fluoroscopy and the radiopaque teflon catheter (1.2/2.0 or 1.5/3 mm) was localized as far caudally and peripherally as possible. In the wedged position the tip of the catheter was generally located 4 cm from the lateral chest wall and in the free position about 4 cm more proximal. Short teflon catheters (1.1/1.4 mm) were also percutaneously inserted into the brachial artery and a peripheral vein in patient and 4 controls another right hepatic vein was usually catheterized from the contralateral arm. In 3 patients and 4 controls a thin polyethylene catheter (PE 60 0.76/1.22 mm) was also percutaneously inserted from cubital vein into the pulmonary artery.

Blood-flow measurement. After a primary dose of about 15 mg ICG (Cardio-Green[®] Hynson Westcott and Dunning, Baltimore Md, USA) a constant infusion of the dye was given at a rate of about 1 mg/min. After an equilibration period of about 30 min 3-5 paired blood samples from the artery and hepatic vein were collected; the arterial sample about 15 sec prior to the venous one. Blood was drawn at generally 5-min intervals at rest during the two work loads and the pause between them if any and after exercise (Fig. 1). The plasma content of ICG was determined on a Hitachi 101 spectrophotometer (Hitachi Ltd Tokyo Japan) after correction for plasma turbidity at 900 nm (20). Hematocrit was determined on all arterial samples by spinning the blood for 5 min at 9000 g in a microhematocrit centrifuge (MB International Equipment Co, Needham, Mass. USA). Plasma and blood osmotes were estimated at rest and 30 min after exercise by injection of about 3 μC ^{51}Cr -human serum albumin. Total hepatic blood flow at rest was calculated by the Bradley technique according to Winkler et al. (36) from the average arterio-hepatic, venous dye difference and the eventual average change in arterial ICG concentration. During and after exercise corrections for variation in arterial dye concentration due to changes in plasma volume were performed mainly according to Halton and Castendorf (11). The hepatic extraction of the dye was measured as the arterio-hepatic venous difference divided by the arterial concentration.

Oxygen saturation and Hb concentration were determined on a CO oximeter (IL IL Lexington, Mass. USA) calibrated against cyanmethemoglobin. Spontaneous oxygen uptake was calculated as total hepatic blood flow \times arterio-hepatic venous oxygen difference. Heart rate was determined from ECG recorded on a VU writer (see below). In 3 patients and 4 controls total oxygen uptake was determined by the Douglas bag technique and macro Scholander analyses and cardiac output and stroke volume were calculated according to the Fick principle.

Table 1 Anthropometric, clinical and laboratory data on the day of the study and their maximal abnormalities prior to the study in 10 patients and corresponding group means for 13 controls

BSA=body surface area, SBl=serum bilirubin, ALP=alkaline phosphatase, Thy=thymol turbidity, RCM=right costal margin

Pat. no.	Age (y)	Height (cm)	Weight (kg)	BSA (m ²)	On the day of catheterization										Liver size palpable below RCM (cm)
					Work load (kpm/min)		Days after onset of jaundice	SBil (mg/ 100 ml)	ALP (U)	LDH (U)	SGOT (U)	SGPT (U)	Thy (U)		
					I	II									
														I	
1	29	177	67	1.85	300	600	15	1.6	6	152	43	146	9	1	
2	26	181	73	1.95	350	700	23	1.8	2.4	184	81	206	11	0	
3	27	182	67	1.89	250	500	14	5.8	4.0	199	320	970	6	1.5	
4	25	186	85	2.11	400	800	12	1.8	2.6	145	170	213	2	0	
5	1	180	62	1.82	400	700	14	1.4	.5	126	36	150	11	0	
6	22	186	72	1.98	600	1 000	10	9.6	2.7	339	710	740	13	2	
7	25	186	80	2.06	500	1 000	22	3.5	2.9	132	79	350	4	2.5	
8	23	185	75	1.90	350	700	15	1.1	3.5	170	78	360	14		
9	25	190	83	2.13	350	700	17	2.6	2.5	134	37	100	1	1	
10	77	169	61	1.73	300	500	16	2.1	2.6	141	88	270	-		
\bar{x}	25.0	182.2	72.7	1.952	340.0	720.0	15.8	3.13	2.83	172	199	350.5	8.1	1	
S.D.	2.4	6.0	8.1	0.130	103.3	175.1	4.0	2.66	0.57	63.3	210.4	284.1	4.7	0.9	
Controls															
\bar{x}	24.8	182.2	74.5	1.973	496.2	984.6	-	0.87	1.84	159.7	18.5	16.9	1.4		
S.D.	2.3	5.3	6.7	0.109	85.3	167.6		0.25	0.34	52.4	4.8	6.3	0.7		
Diff.	8.2	0.0	-1.8	-0.021	-116.2	-264.6	-	2.26	0.99	12.5	140.7	333.6	6.7		
P	>0.80	>0.99	>0.50	>0.60				>0.50			

Maximal abnormalities prior to catheterization

1	6	8.5	4.8	254	310	1 000	17	2
2	6	11.7	3.8	237	202	1 000	3	1
3	6	11.9	5.2	466	1 000	000	11	3
4	6	3.3	4.3	47	780	570	2	0
5	5	5.4	4.9	411	880	1 000	6	1
6	6	12.2	3.1	777	780	1 000	23	3
7	1	14.4	7.5	546	1 000	1 000	20	4
8	2	4.3	4.7	579	1 000	2 000	11	3
9	8	16.6	3.8	290	950	1 000	15	2
10	2	10.7	5.2	499	1 300	2 700		3
\bar{x}	5.2	9.90	4.73	380.6	820.2	1 377.0	14.3	
S.D.	1.9	4.42	1.19	134.3	337.2	667.1	7.3	1.1

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ where p is the probability that the difference between the groups was caused by random factors. Upper normal limits given below each biochemical liver test.

Wilcoxon's nonparametric testing (28).

Total blood loss did not exceed 250 ml, except in the last 4 patients and controls, in whom 100 ml extra blood was drawn for metabolic studies.

Intravascular pressures were measured by transducers (Bell & Howell no. L 221 Woking, Surrey England) using an EMMA amplifier (SE labs, Feltham Middlesex, England) and UV writer (SE 3006, SE labs). Reference point for zero pressure was the mid-thoracic level at the insertion of the fourth rib on the sternum. Free and wedged hepatic venous pressures were repeatedly recorded at rest during quiet breathing with large amplifications. To check whether or not properly wedged catheter position was obtained, the un-

damped and damped curve was analysed. For each measurement the pressure of the damped curve was integrated manually over 20-30 sec and related to the nearest 1/10 mmHg (16).

Prior to blood sampling the arterial BP was similarly recorded. Splanchnic flow resistance was derived as the fall in pressure (i.e. mean arterial BP minus free hepatic venous pressure) divided by total hepatic blood flow. Postarrest flow resistance was derived as the fall in pressure from wedged to free hepatic vein divided by total hepatic blood flow.

Biochemical liver tests. Serum bilirubin, SGOT, SGPT, LDH, alkaline phosphatase and thymol turbidity

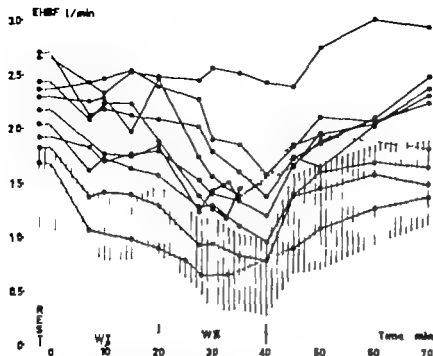


Fig. 1 Estimated hepatic blood flow (EHBF) at rest (O) during two separate bicycle work loads (W1 and W2), and after exercise (●) in the patient. — discontinued work load, hatched area = range of the controls.

were determined by standard methods on blood sampled at rest before the flow study. These tests were also performed on blood sampled 30 min after exercise on the 3rd or 4th and on the 6th day after catheterization

and then once a week until the patient was discharged and generally at 3–4 week intervals until their normalization.

Statistical calculations were made by standard methods (28). The following probability levels of significance were used: $p < 0.001$ highly significant, $p < 0.01$ significant and $p < 0.05$ probably significant.

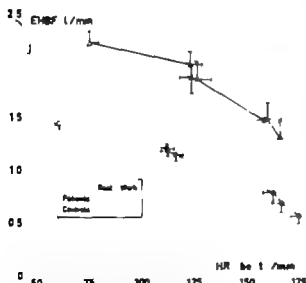


Fig. 2 Estimated hepatic blood flow (EHBF) at rest (unfilled symbols) after 10 and 15 min and at the end of two generally 20 min periods of supine leg exercise (filled symbols) in relation to heart rate (HR) in the patients (Δ — Δ) and the controls (O—O). Group means \pm S.E.M. No. of individuals is given when recorded.

RESULTS

The patients were on an average studied at a heaviest work load of 720 kpm/min and a heart rate of 166 beats/min, as compared with 985 kpm/min and 175 beats/min for the controls. The difference in work load was significant but not the difference in heart rate. In relation to work load the patients had a heart rate about 15 beats/min higher than had the controls. Total blood volume was equal in patients and controls on an average 4.27 and 5.24 l respectively. Due to exhaustion 4 patients and 7 controls had to terminate the second work load after 10–19 min, and on an average this work load lasted for 18.75 and 17.60 min for patients and controls respectively. For both groups final values are given for this work load. The change in heart rate during the heaviest load was the same in both groups, 8–14 beats/min from the 10th min to the final value (Fig. 2).

Table II Some circulatory data at rest (RI) at the end of the first (WI) and the second work load (WII) and 30 min after exercise (RII) in the patients and controls

	Estimated hepatic blood flow (l/min)				Arterio-hepatic venous oxygen difference (ml/l)				Splanchnic oxygen uptake (ml/min)			
	RI	WI	WII	RII	RI	WI	WII	RII	RI	WI	WII	RII
Patients												
\bar{X}	1.1	1.87	1.30	2.07	36.1	45.7	74.9	42.9	79.7	82.6	89.8	87.4
S.D.	0.34	0.57	0.51	0.57	4.8	8.8	25.3	8.0	16.0	70.	28.9	1.4
	10	10	10	9	10	10	10	9	10	10	10	9
Controls												
\bar{X}	1.44	1.15	0.56	1.68	43.0	56.9	130.9	46.4	60.9	65.4	67.1	66.8
S.D.	0.21	0.22	0.21	0.3	7.8	10.9	34.7	8.7	9.	15.9	12.9	12.9
	13	13	1	12	12	1	11	1	1	1	11	11
Difference	0.77	0.77	0.74	0.59	-6.9	-11.2	-56.0	-3.5	18.8	17.2	-7	20.6
<i>p</i>	**		***				**	>0.30				
	Heart rate (beats/min)				Mean arterial BP (mmHg)				Splanchnic flow resistance (mmHg/l/min)			
	RI	WI	WII	RII	RI	WI	WII	RII	RI	WI	WII	RII
Patients												
\bar{X}	75.2	125.9	163.6	96.5	88.3	92.2	98.6	84.8	37.5	50.8	87.6	40.9
S.D.	14.0	19.9	21.8	14.6	10.0	6.7	3.9	10.5	8.9	23.6	41.3	14.6
	10	10	10	10	10	10	10	10	10	10	10	9
Controls												
\bar{X}	60.6	116.1	175.0	82.8	90.7	100.8	110.	90.5	60.0	83.8	204.5	57.0
S.D.	7.9	13.1	14.6	10.8	9.6	4.9	7.4	8.4	9.7	17.5	94.9	10.
	13	13	13	13	12	13	12	11	1	13	11	9
Difference	14.6	9.8	-9.4	13.7	-2.4	8.6	-11.6	-5.7	-22.5	-33.0	-11.9	-16.1
<i>p</i>	**	>0.10	>0.20		>0.40		**	>0.10			**	

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ where *p* is the probability that the difference between the groups was caused by random factors

Hepatic blood flow The hepatic circulation at rest in the patients was characterized by larger total hepatic blood flow, lower splanchnic flow resistance, lower arterio-hepatic venous oxygen difference and higher splanchnic oxygen uptake than in the controls (Table II). Neither in patients nor in controls was total hepatic blood flow related to any of the body-size variables. At the highest work load the hepatic blood flow of the patients had decreased to an average of 59% of the value at rest as against 39% in the controls. The hepatic blood flow in relation to heart rate as a measure of the relative work load is given for both groups in Fig. 2. When the reduced hepatic blood flow during exercise was expressed in % of the value at rest the patients and the controls had the same flow reduction in relation to the increase in heart rate (Fig. 3). Although the mean arterial BPs during exercise were significantly

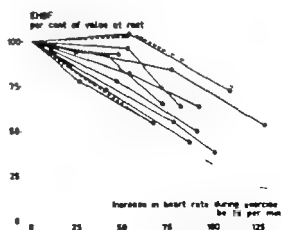


Fig. 3 Change in estimated hepatic blood flow (EHBF) during exercise (end of two generally 20 min periods of supine leg exercise) in relation to change in heart rate in the patients. The area inside the broken line—range of the controls.

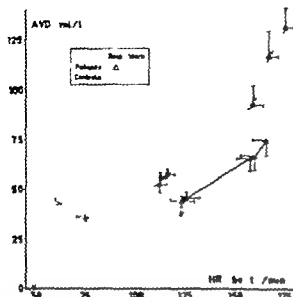


Fig. 4. Arterio-hepatic venous oxygen difference (A/D) at rest (unfilled symbol) after 10 and 15 min and at the end of two generally 70 min periods of supine leg exercise (filled symbols) in relation to heart rate (HR) in the patients and controls. Group mean \pm S.E.M. No. of individuals given when reduced. Symbols as in Fig. 1.

lower in the patients than in the controls (Table II) the increases in splanchnic flow resistance with exercise were not significantly different when related to the simultaneous increases in heart rate.

At 30 min after exercise all variables except heart rate returned to the initial values at rest in both groups. The heart rates being elevated about 70 beats/min ($p < 0.001$) (Table II).

Hepatic circulations. In the patients the average hepatic venous oxygen saturation was reduced from 79% at rest to 74 and 60% respectively at the end of the first and second work loads. The corresponding values for the controls were 76% at rest and 70 and 36% during exercise. The resultant lower arterio-hepatic venous oxygen difference at rest in the patients as compared with the controls was still more marked during exercise (Table II, Fig. 4). At 30 min after exercise the hepatic venous oxygen saturation and the arterio-hepatic venous oxygen difference had not completely returned to the resting values in the patients ($p < 0.01$) in contrast to the controls ($p > 0.10$). The average splanchnic oxygen uptake at rest was significantly higher in the patients than in the controls (Table II). In neither group was the

splanchnic oxygen uptake during or after exercise significantly changed from the resting level, indicating that the increase in arterio-hepatic venous oxygen difference during exercise compensated the decrease in blood flow. Splanchnic oxygen uptake at rest as a fraction of total oxygen uptake was on an average 0.34 (range 0.30–0.37) in 3 patients and 0.25 (range 0.20–0.29) in 4 controls.

The hepatic extraction of ICG at rest was 54 (range 37–75)% in the patients and 75 (range 65–89)% in the controls. The difference between the groups being significant. Among the patients those with high serum bilirubin values had the lowest extraction ($r = -0.856$).

Hepatic venous pressure. The average wedged hepatic venous pressure at rest in the patients was not significantly different from that in the controls (Table III). Also the free hepatic venous pressure was similar in patients and controls both before and after exercise. In both groups wedged and especially free hepatic venous pressure decreased during exercise. During the 15-min pause between the work loads the average wedged hepatic venous pressure was unchanged as compared with that before exercise for both patients and controls. The average difference between wedged and free hepatic venous pressure was significantly higher at rest in the patients as compared with the controls (Table III). In both groups the pressure difference increased after exercise, but this change was significant for the controls alone ($p < 0.01$). After the first work load the pressure difference was 3.4 mmHg in the patients and 1.1 mmHg in the controls.

Postsinusoidal flow resistance at rest was significantly different between patients and controls on an average 1.30 and 0.69 mmHg/l/min respectively.

Relationships between hepatic circulation and laboratory and clinical data. were studied. In the patients total hepatic blood flow, arterio-hepatic venous oxygen difference, duration after onset of jaundice and liver size were not significantly related to any of the variables. Relationships of significance are given in Table IV. In the controls the only correlation of any significance was between the serum bilirubin and the difference between wedged and free hepatic venous pressure ($r = 0.645$).

Effect of exercise on symptoms and biochemical liver tests. In the 8 patients who at the time of

Table III Wedged hepatic venous pressure (WHVP) and the difference between wedged and free hepatic venous pressure (W-FHVP) at rest (RI) and after the second work load (RII) in 9 patients and 11 controls

	WHVP (mmHg)			W-FHVP (mmHg)			
	RI	RII	RII-RI	RI	RII	RII-RI	RII/RI
Patients							
\bar{x}	10.1	9	-0.9	9	3.4	0.6	1.2
S.D.	2.2	1.8	0.9	1.1	1.6	0.9	0.4
Controls							
\bar{x}	8.7	7.0	-1.7	0.9	1.9	1.0	1.1
S.D.	1.7	1.8	1	0.5	0.7	0.9	0.6
Difference	1.4	2	0.8	0.0	1.5	-0.4	-1.6
p	>0.10		>0.10	**		>0.30	>0.05

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ where p is the probability that the difference between the groups was caused by random factors.

the study had serum bilirubin below 4 mg/100 ml and SGOT and SGPT below 150 and 400 U (Table I) no negative effects of the exercise were noted on recovery or relapse rate. Of the other patients no 3 showed a normal recovery after exercise and the values of his liver tests were lower on the 4th day than before catheterization. On the 6th day without aggravated symptoms a slight and temporary increase was noted for SGOT from 300 to 390 U whereas SGPT and serum bilirubin were unchanged. Thereafter all the liver test values decreased and the recovery was un-

eventful. In patient 6 serum bilirubin decreased to 8.4 mg/100 ml on the 4th day but SGOT and SGPT both rose to 1000 U. On the 6th day the corresponding values were 71 960 and 1000. On the 10th day the patient experienced malaise (fatigue, loss of appetite and increased jaundice). On the 11th day serum bilirubin had increased to 11.3 mg/100 ml whereas SGOT and SGPT had decreased to 700 and 840 U respectively. One week later he began to improve clinically in spite of a serum bilirubin value of 13.1 mg/100 ml. SGOT and SGPT were down to 460 and 490 U respectively. Thereafter serum bilirubin decreased and the recovery was uneventful.

Table IV Relationships between hepatic circulatory and laboratory data in the patients and the total material

Correlation coefficient is only given when at least probability significance was observed.
SBl=serum bilirubin, W-FHVP=difference between wedged and free hepatic venous pressure

	Splanchnic = splanchnic = splanchnic	W-FHVP	Total hepatic blood flow
Patients			
SBl	0.814*	0.890*	n.s.
SGOT	0.838*	0.883	s.
SGPT	0.853**	0.666	n.s.
W-FHVP	0.761		
Total material			
SBl	0.793**	0.866**	0.827*
SGOT	0.783	0.805	0.571**
SGPT	0.835	0.803	0.749*
W-FHVP	0.717*		0.669*

$p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.s.=not significant.

DISCUSSION

As the data of the controls were in good agreement with results obtained by others (3, 26, 33, 34, 36) both at rest and during exercise the changes in the patients were evidently related to the disease. This is reinforced by the observation of normal hepatic circulation at reexamination of 3 patients 5 weeks after the present study (15).

The patients were studied on an average at lower work loads than the controls, mostly due to lower physical working capacity. However the pertinent comparisons during exercise between patients and controls were made in relation to heart rate as a measure of the relative work load since the decrease in hepatic blood flow during exercise has been found to be more closely related to rela-

tive than to absolute work load (26). Thus the present differences in work load between patients and controls are of minor importance. At re-examination about 5 weeks later of 3 of the present patients (15) their heart rates at rest and during exercise were on an average 13–20 beats/min lower than during the first study. Therefore the higher heart rates during exercise of the patients as compared with the controls seem to have been caused by the disease or its treatment with bed rest and not by selection or differences in pre-morbid physical capacity. The experimental conditions varied slightly as in 7 of 10 patients and 4 of 13 controls a 15-min rest was inserted between the two work loads. In 6 of the patients and 9 of the controls a light breakfast was also allowed 2–3 hours prior to the flow study whereas the others were studied after overnight fast. By group comparison however no effects of these variations of any significance were observed on the variables studied or their variation with exercise.

Blood is generally sampled from only one hepatic vein on the assumption that this sample is representative of all hepatic venous blood but variations in dye extraction have been observed with catheters positioned in different right hepatic veins (4). In one patient with moderate liver cirrhosis and 7 subjects without liver disease (36) significant differences in extraction between the two usually right-sided hepatic venous catheters seen in about 5% of the determinations but usually only for one or two of the 5 substances.

Great simultaneous regional differences in extraction of ICG and oxygen have been noted in alcoholics with marked cirrhosis of the liver (30) indicating heterogeneity of metabolic activity in relation to blood flow perhaps related to regional variations in cell regeneration (31). In the present study tests for the occurrence of gross heterogeneity of hepatic circulation were made in 2 patients and 5 controls by simultaneous sampling from an additional catheter located in a more cranial right hepatic vein both at rest during and after exercise. However there was no difference of any significance between the two hepatic veins with respect to ICG concentration or oxygen saturation. Thus hepatic blood flow and oxygen extraction seem to be similar in different parts of the right liver lobe at least in areas of this size which is in agreement with the finding of no gross heterogeneity in the distribution of ^{201}Au in

photoscans of 8 patients with viral hepatitis (21).

The hyperkinetic hepatic circulation was not part of a general vasodilatation. In 3 patients and 4 controls cardiac output was determined simultaneously with hepatic blood flow. The patients had a mean cardiac output at rest of 8.7 l/min and a mean hepatic blood flow of 2.6 l/min, or 30% of the cardiac output. The corresponding values for the controls were 7.3 and 1.5 l/min and 20% respectively. The average flow of blood to extrahepatic parts of the body was thus the same in both groups.

The high hepatic blood flows in acute infectious hepatitis agree with the hyperemia in the diseased organ in many other infections. However in previous studies (6, 19, 21, 24, 27) no flow increase was observed although in one of them (21) the reliable method with constant infusion of ICG was used. In earlier studies only the hepatic mesenchymal clearance of radioactive gold (6, 19) and chronic phosphate (27) was measured which is not necessarily related to hepatic blood flow.

It has been shown in man that total hepatic blood flow could increase markedly after typhoid vaccination even when fever was prevented (3). In rabbits an increased clearance of ICG was observed for many weeks after i.v. injections of heterologous protein (14) which was considered to be due to an induced stimulation of the parenchymal cells, may be by a higher blood flow. Our present data do not allow any conclusions as to whether or not immunological factors contribute to the observed high hepatic blood flows in acute infectious hepatitis.

A more probable cause of the high hepatic blood flow might be the hepatic cellular damage and regeneration unevenly distributed within the lobules. A local vasodilatation evoked by such changes can only be mediated by the hepatic artery as a decrease in the small portal venous flow resistance over the liver is unlikely and anyhow cannot measurably increase the portal venous blood flow. On the contrary an increased flow resistance over the portal sinusoids should be expected as in fatty liver and toxic hepatitis (22), where the swollen hepatocytes compress the sinusoids, increase the sinusoidal flow resistance and impair the local blood flow. If the hepatic blood flow in infectious hepatitis is similarly unevenly impaired within the lobules anoxia will develop

in some areas. A resultant metabolic vasodilatation will correspond to the most anoxic regions rather than to the average need and the flow increase in excess of the metabolic demands will result in a lower arterio-hepatic venous oxygen difference and a higher oxygen saturation in the hepatic venous blood as was found in the present study.

The increased difference between wedged and free hepatic venous pressure in the present patients as compared with the controls was explained by the increased postsinusoidal flow resistance possibly due to cellular swelling, but altered microcirculation with increased hepatic artery flow might also have contributed. Although the absolute value of the difference between patients and controls was low it corresponded to a ~ 3 times higher flow resistance. Despite these significant changes the wedged hepatic venous pressures were not significantly higher in the patients than in the controls which findings are in good agreement with those of Preisig *et al.* (21). The signs of increased portal venous pressures in viral hepatitis reported in other materials (9-13) probably only indicate somewhat more marked changes than in the present study.

There were significant correlations between the difference between wedged and free hepatic venous pressure and the state of the disease as estimated by biochemical liver tests even if only small absolute differences were noted between patients and controls before and after exercise.

The indications of larger than normal oxygen consumption during regeneration of hepatic tissue (5) might explain the present observation that both splanchnic oxygen uptake and blood flow were decreased in our patients the biochemical liver tests were more closely correlated to the splanchnic oxygen uptake than to the total hepatic blood flow that is more influenced by other factors e.g. the sympathetic activity at the time of the study.

In the last 4 patients and controls blood samples were analysed for studies of carbohydrate, protein and fat metabolism (Lundbergh, Furst, Strandell and Wahren personal communication 1974), but so far no major abnormalities have been observed. The high splanchnic oxygen uptake of the patients could have been caused by an increased hepatic combustion of fat which increases the splanchnic oxygen uptake compared with the situation when

predominant carbohydrate metabolism occurs (12). However in the present material there was no significant difference in respiratory quotient between 3 patients and 4 controls: the average value being 0.87 (range 0.84-0.94) for the patients and 0.77 (range 0.70-0.92) for the controls. The dietary and medical treatment given the patients cannot have affected the hepatic circulation significantly: only once or twice a week was the normal hospital food replaced by a meal less rich in fat and the only medical treatment given was vitamins orally twice the daily need.

The flow increase can have been related to cellular changes and to immunological responses. In both instances the hepatic artery blood flow should be increased but an immunological response should also include an increased splenic blood flow. No data on the distribution of splanchnic blood flow in viral hepatitis are yet at hand but methods to separate these flows in awake patients are now available (29). They necessitate the insertion of multiple portal venous catheters (35) and have not been used so far. Indirect evidence of an increased hepatic artery blood flow was however present in our patients since in one case a wedged hepatic venous pressure was recorded after exercise with one marked wave resembling an arterial curve and a pressure level close to the arterial one (17). In others unstable wedged pressure recordings were seen after exercise with sliding pressures up to 30-40 mmHg which cannot represent portal venous pressure.

In spite of great differences in flow values the decrease in hepatic blood flow during exercise was equal in both groups in relation to the increase in heart rate indicating the same sympathetic flow control in both groups.

During and after the second world war epidemics of infectious hepatitis occurred in many countries and some of the patients treated in military hospitals constituted materials for studies regarding therapy. The importance of strict bed rest and high protein intake in viral hepatitis was stressed (1). This opinion was unchallenged until other authors (7-14) reported that strict bed rest was not superior to bed rest dilatation after the acute phase. Deleterious effects of vigorous exercise during the first days of the preicteric phase have been observed in one study (13): 3 of 5 patients died in hepatic coma within 4-16 days after onset of disease but no figures were reported

of how often physical exercise was performed during the preicteric stage. To establish when heavy exercise may be allowed without risk in viral hepatitis patients have been submitted to exercise. In Vietnam 398 American servicemen with infectious hepatitis were included in such a study (25). Half of them were ambulant in the ward without prescribed exercise whereas the other half participated in daily exercise for 3 hours as soon as their symptoms were considered slight. Average serum bilirubin was 7.5 mg/100 ml. No adverse effects of the exercise were noted on recovery or relapse rate. However neither the material nor the intensity of the work was clearly defined. These findings were reinforced by a study in 23 patients with acute viral hepatitis who performed 1 min of moderately heavy bicycle exercise for 11 consecutive days (8).

In the present material none of the 8 patients with values of serum bilirubin below 4 mg/100 ml SGOT and SGPT below 150 and 400 U at the time of the catheterization showed any signs or symptoms of negative effects of the exercise. Of the patients with serum bilirubin values of 5.8 and 9.6 mg/100 ml respectively, no 3 showed a slight delay in the decrease of the liver-test values and no 6 a relapse that delayed his recovery for about 2 weeks. Patient 3 performed only 400 kpm/min with a final heart rate of 170 beats/min, whereas patient 6 was well trained and performed a heaviest load of 1000 kpm/min with a final heart rate of 180 beats/min. These prolonged recoveries may have been due to chance as this clinical course occurs in about 10% of patients with acute viral hepatitis according to current textbooks and the experience in this hospital. However the possibility cannot be excluded that the physical exercise may have been the provoking factor and pending more extensive studies heavy physical exercise should be avoided until after the acute phase, e.g. when serum bilirubin is down to about 4 mg/100 ml.

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Lp(a) LIPOPROTEIN AND PRE β_1 LIPOPROTEIN IN YOUNG ADULTS

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Abstract A very strong association between phenotype Lp(a+) and presence of pre- β_1 -lipoprotein has been observed in a series of healthy young adults. The data are in agreement with the view that pre- β_1 -lipoprotein is identical to the lipoproteins carrying the Lp(a) antigen, the Lp(a) lipoprotein. Lp(a) antigen/pre- β_1 -lipoprotein occurred more frequently in persons with positive than with negative family history of coronary heart disease, though this trend was not statistically significant. Occurrence of Lp(a) antigen/pre- β_1 -lipoprotein was not correlated with smoking or with the use of contraceptive pill.

samples studied previously. Since both lipoprotein traits are present in a certain proportion of normal sera, they are not the results of disease. They may however represent genetic risk factors with respect to CHD, demonstrable early in life.

The purpose of the present investigation was to study whether the two phenomena are associated also in healthy young people and to study the possible relationship between these phenomena and some other factors which may be related to the development of CHD.

The Lp(a) antigen is a genetically determined trait of human serum lipoprotein (1). The antigen as defined by standardized techniques and antisera in our laboratory is present in about 35% of healthy people of Western European extraction and it resides in a distinct species of lipoprotein molecules, the Lp(a) lipoprotein. This lipoprotein migrates faster than β -lipoprotein but slower than pre- β -lipoprotein in agarose or paper electrophoresis and it is present in the 1.050-1.080 g/ml density class upon ultracentrifugation.

Pre- β_1 -lipoprotein is an atypical pre- β -lipoprotein which is present in a certain proportion of normal sera. It occurs relatively frequently in the sera of patients with coronary heart disease (CHD). When pre- β_1 -lipoprotein is present, it occurs as a distinct electrophoretic zone in the area between β -lipoprotein and regular pre- β -lipoprotein. The presence of pre- β_1 -lipoprotein appears to be under genetic control (4).

A study of Finnish patients with suspected or proven CHD (3) showed that a very close relationship exists between the phenotype Lp(a+) and the occurrence of pre- β_1 -lipoprotein in the serum. Both phenomena were more frequent in a sample of Finnish CHD patients than in normal population

MATERIAL AND METHODS

Blood samples were collected from two series of healthy young Swedes from the Bodø area.

The first series consisted of 75 males, 16-17 years old. The blood samples were drawn in the morning after 12 hours fast and centrifuged after clotting. Serum was then secured immediately chilled, stored at 4°C and subjected to electrophoretic and other clinical-chemical analyses as described previously (5). A portion of each serum sample was frozen immediately and transferred by air without delay in the frozen state to the Institute of Medical Genetics, University of Oslo, where Lp typing was performed.

The second series consisted of 53 females, 4-30 years old. The blood samples from these persons were taken under less strict conditions. The samples were kept for several hours at 4°C before freezing and were stored at -20°C in Bodø for several weeks before they were shipped to the Institute in Oslo for Lp typing.

Both males and females were asked to complete questionnaire about their smoking habits. The females also answered a questionnaire aimed at revealing CHD and high BP among their relatives and were questioned with respect to use of oral contraceptives. Because of the young age of the subjects, family anamnesis was concentrated on disease in the women's parents, grandparents, uncles and aunts.

Scoring with respect to the Lp(a) antigen was conducted in Oslo as described previously (1). Electrophoretic lipoprotein analysis and all other laboratory

Table 1 Distribution of 75 young healthy males and 51 young healthy females with respect to Lp phenotype and pre- β_2 -lipoprotein

		No. of individuals			χ^2	p
		Lp(a+)	Lp(a-)	Total		
Male						
Lp(a) antigen and pre- β_2 -lipoprotein	Pre- β_2 -lipoprotein present	17		17	40.60	<0.0001
	Pre- β_2 -lipoprotein absent	5	51	56		
	Total	22	53	75		
Lp(a) antigen and high amount of pre- β_2 - lipoprotein	Samples with strong pre- β_2 -lipoprotein band	6	0	6	12.22	<0.001
	All other samples	16	53	69		
	Total	22	53	75		
Female						
Lp(a) antigen and pre- β_2 -lipoprotein	Pre- β_2 -lipoprotein present	9	7	16	70.19	<0.0001
	Pre- β_2 -lipoprotein absent	0	35	35		
	Total	9	42	51		

analyses were carried out as Boden *et al* reported previously (5). Scoring with respect to Lp(a) phenotype and presence or absence of pre- β_2 -lipoprotein was carried out independently and blindly.

RESULTS

Relationship between Lp(a) antigen and pre- β_2 -lipoprotein

In the Lp typing procedure the samples from healthy young males yielded clear-cut results every sample being scorable as positive or negative with respect to the Lp(a) antigen. Of the 75 samples 22 gave a positive reaction. The reactions with 4 of the sera were weak but clearly visible. It was concluded that Lp typing in this series probably yielded valid results.

Lp typing was more difficult in the series of young females. Of 53 serum samples only 9 were clearly positive of which yielded very weak reactions. The reactions with the remaining 7 positive samples were clearly weaker than the reactions usually observed with fresh sera. Two of the 53 sera could not be assigned either to positives or negatives and the results with these were omitted in the further analyses. The fact that sera had not been treated in a way optimal for Lp typing, the low frequency of positive sera in this sample, the faint precipitin bands observed and the 2 doubtful reactions made it reasonable to assume that several truly Lp(a+) sera might have been typed

as Lp(a-). Since it seemed reasonable to assume that the sera which still reacted positively were those which originally had been strongly Lp(a+), it was considered of interest to analyse this series of samples as well despite the fact that the Lp(a+) frequency observed in this sample might be significantly lower than the true frequency.

The distribution of the 75 young healthy males and the 51 females with respect to Lp(a) phenotype and presence or absence of pre- β_2 -lipoprotein is shown in Table 1. Among the young males scored as pre- β_2 -negative there were some whose sera exhibited extremely weak and doubtful zones upon agarose gel electrophoresis. In a previous comparison with different other parameters (5) these subjects were classified as pre- β_2 -lipoprotein positive.

In both series a highly significant, positive correlation between the two phenomena was observed ($p < 0.0001$). Among the males, except for 2 sera scored as possessing pre- β_2 -lipoprotein the immunological Lp test revealed all sera with this lipoprotein and 5 more. All Lp(a+) sera in the female series exhibited pre- β_2 -lipoprotein. A visual scoring of the intensity of the pre- β_2 -lipoprotein zone was performed and 6 samples were scored as strongly positive with respect to pre- β_2 -lipoprotein, presumably containing a high amount of this lipoprotein. Table 1 also shows the distribution of the 75 young males with respect to high amount of pre- β_2 -lipoprotein and Lp(a) phenotype. Again a

Table II *Lp phenotype and pre- β -lipoprotein in young healthy women with positive or negative family history of CHD*

A=family history negative B=family history positive C=CHD in one of the parents
 Figures within parentheses denote percentages

	A (n)	B (n)	A vs. B		C (n)	A vs. C	
			χ^2	P		χ^2	P
Lp(a)+	1/14 (7)	6/30 (20)	0.41	0.50-0.70	3/13 (23)	0.39	0.50-0.70
Pre- β -lipoprotein present	1/14 (14)	1/13* (37.5)	1.50	0.20-0.30	6/13 (46)	1.93	0.10-0.20

high degree of association is obvious ($p < 0.001$). All samples scored as strongly positive with respect to the pre- β -lipoprotein were also positive (and exhibited strong reactions) with respect to the Lp(a) antigen.

Lp phenotype and pre- β -lipoprotein in relation to family history of coronary heart disease

Forty-six of the females completed the questionnaire concerning familial occurrence of CHD (among them were the whose sera could not be scored with respect to Lp phenotype). Of these females 14 had a completely negative family history. Thirteen reported coronary artery disease in at least one of the parents (and often in several other relatives). For the analysis no distinction was made between sustained myocardial infarction and angina pectoris. Two of the females each reported high BP in one of the parents but no evidence of myocardial infarction or CHD. The remaining 17 reported one or more cases of coronary artery disease among grandparents, uncles and aunts but no such disease in their parents. Table II summarizes the data with respect to Lp phenotype and presence or absence of pre- β -lipoprotein in females with negative or positive family history. The Lp(a) frequency was 1.8 times higher in females with a positive family history than in those with a negative and pre- β -lipoprotein could be demonstrated 2.6 times more frequently in those with a positive family history than in those with a negative. However these differences were not statistically significant.

Table II shows also a comparison between females with a negative family history and those who reported CHD in one of their parents. The tendency to a higher frequency of positives for the Lp antigen as well as the pre- β -lipoprotein is more pronounced in this group of females who

have a first-degree relative with CHD but the difference from those with a negative family history is still not significant.

Lp phenotype and pre- β -lipoprotein in relation to use of contraceptive pills

In Table III the material of young females is analysed with respect to use of contraceptive pills and Lp(a) lipoprotein or pre- β -lipoprotein. No association between use of contraceptive pills and the occurrence of the lipoprotein phenomena was revealed.

Lp phenotype and pre- β -lipoprotein in relation to smoking

In Table IV the series of young females and males are analysed with respect to smoking habits. Lp phenotype and presence or absence of pre- β -lipoprotein. For the purpose of the analyses persons smoking 10 or more cigarettes per day were scored as smokers and those smoking less as non-smokers.

No association between Lp phenotype and smoking or between presence of pre- β -lipoprotein and smoking was found in the females. Only 5 of

Table III *Lp phenotype and pre- β -lipoprotein in young healthy women using or not using the pill*

Figures within parentheses denote percentages

	Not using the pill (n)	Using the pill (n)		
			χ^2	P
Lp(a) +	7/33 (21)	1/18 (11)	0.77	0.50-0.70
Pre- β -lipoprotein present	11/35 (31)	6/18 (33)	0.03	0.80-0.90

Table IV *Lp phenotype and pre- β_2 -lipoprotein in young healthy females and males smokers or non-smokers*

Figures within parentheses denote percentages

	Smokers (n)	Non-smokers (n)	χ^2	P
<i>Females</i>				
Lp(a)	2/16 (12.5)	5/28 (18)	0.002	0.95-0.98
Pre- β_2 -lipoprotein present	5/17 (29)	9/29 (31)	0.05	0.80-0.90
<i>Males</i>				
Lp(a)	2/7 (29)	14/46 (30)	0.1	0.70-0.80
Pre- β_2 -lipoprotein present	2/7 (29)	22/46 (48)	0.30	0.50-0.70

the 29 female non-smokers smoked 1-9 cigarettes per day. The conclusions from the association tests were not altered by including those 5 in the smoking group instead of scoring them among the non-smokers ($0.80 < p < 0.90$ for both comparisons).

Similarly in the series of young males there was no association between smoking and either Lp(a) phenotype or presence of pre- β_2 -lipoprotein. This conclusion was not altered by including in the smoker group those who smoked 1-9 cigarettes per day ($0.50 < p < 0.70$ for the Lp comparison and $0.70 < p < 0.80$ for the pre- β_2 -lipoprotein comparison).

DISCUSSION

The results of Lp typing of the sample of young males from Northern Sweden revealed a frequency of the phenotype Lp(a+) in this sample of 29.33%. This is not significantly different from the frequency in a sample of healthy blood donors in the Oslo area ($\chi^2=0.81$, $0.30 < p < 0.50$). The sample of North-Swedish females is not suitable for Lp(a) frequency determination since there is evidence that some positive reactions were lost. Weak reactions with sera which type as Lp(a+) and a low frequency of positive reactors are typical findings in Lp typing of sera which are not sufficiently fresh for this analysis. Presumably the sera of this sample which still reacted positively were those possessing a high amount of Lp(a) antigen and this was the rationale for utilizing also this series of persons for the comparisons in the present investigation.

The comparative analysis of the distribution of Lp(a) antigen and pre- β_2 -lipoprotein in the sample of 75 young healthy males (Table I) proves beyond any reasonable doubt that there is a strong, positive association between the presence of the Lp(a) antigen and the pre- β_2 -lipoprotein ($p < 0.0001$).

Of the 7 samples which were neither positive nor negative with both the immunological and the electrophoretic techniques 5 were Lp(a+) and lacked the pre- β_2 -lipoprotein. This is in agreement with the view that more positives are revealed by the more sensitive immunological technique. The remaining 2 sera were Lp(a-) but exhibited a distinct electrophoretic band in the pre- β_2 -region. We have previously commented on the possible VLDL nature of electrophoretic bands in this region, occurring with Lp(a-) serum. However no ultracentrifugal characterization of the lipoproteins was done in this study.

This high degree of association between the two lipoprotein phenomena confirms the association observed in a sample of Finnish patients with suspected or proven CHD (3). The fact that three different samples, the Finnish series and the two included in the present study, have all revealed a highly significant association between the Lp(a) antigen and presence of pre- β_2 -lipoprotein provides firm evidence that the two lipoprotein phenomena are indeed very closely related. The results clearly show that the pre- β_2 -lipoprotein may be identical to the distinct lipoprotein population carrying the Lp(a) antigen, the Lp(a) lipoprotein.

Although the statistical analysis for a possible correlation between Lp(a) antigen and pre- β_2 -lipoprotein on the one hand and a family history of CHD on the other (Table II) did not yield significant χ^2 values, it seems noteworthy that the tendency in the present series is very similar to that in the Finnish patients (3). In the series of young females, those with one parent with CHD presumably represented the highest degree of familial tendency to this disease. When this group of females was compared with those with a completely negative family history, it was striking that a 3:1 relationship was observed for the frequency of Lp(a+) as well as for presence of pre- β_2 -lipoprotein (Table II). Pre- β_2 -lipoprotein/Lp(a) lipoprotein is presumably closely related to the lipoprotein component observed by Knoblock and Hall (7) and their component appeared to be pos-

tively correlated with a family history of CHD. Thus a trend in association with familial aggregation of CHD has been observed in total of three samples: our Finnish sample (3), the present sample of young females and the sample of healthy persons reported by Knoblock and Hall. Since all three series were small, the conclusions must still be drawn with caution. Nevertheless the combined results do suggest that Lp(a) antigen and pre- β -lipoprotein are positively correlated with familial aggregation of CHD. These genetically determined lipoprotein phenomena may therefore constitute important genetic risk factors with respect to CHD.

As far as the present investigation goes, there appears to be no correlation between the use of contraceptive pills and the presence of Lp(a) antigen or pre- β -lipoprotein (Table III).

No association between smoking and presence of either of the two lipoprotein phenomena under study was demonstrable in the two present samples (Table IV). This finding differs from the results in a Finnish study (6) where an association between smoking and presence of pre- β -lipoprotein was found. In the total Finnish sample no association between smoking and the phenotype Lp(a+) was demonstrable (3). When the Finnish patients who had radiologically demonstrable CHD were analysed separately, there was an association between smoking and the phenotype Lp(a+). Among those without radiologically demonstrable abnormalities there was no suggestion of an association.

The two samples analysed in the present study provide evidence that neither Lp(a) antigen nor pre- β -lipoprotein is associated with smoking in young healthy adults. The significance of the as-

sociation between the two lipoprotein phenomena and smoking in patients with manifest CHD is at present unknown, and there is a need for further study of this apparent association. It is, however, tempting to speculate that the association observed in the patients may imply that those possessing the Lp(a) antigen/pre- β -lipoprotein and who are smokers run a increased risk of contracting manifest CHD.

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CUTANEOUS ERYTHEMA WITH OR WITHOUT NECROSIS LOCALIZED TO THE LEGS AND FEET—A LESION IN ELDERLY DIABETICS

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Abstract. The paper describes cutaneous lesions of the leg and/or foot in elderly diabetics, consisting of areas of erythema the size of child's palm or larger and in the same group of patients areas of necrosis from the size of corns up to the size of child's palm surrounded by erythema. The necrosis involves all layers of the skin. Similar skin lesions are seen in elderly persons without recognized diabetes. When a group of patients of the latter type was compared with a control group it appeared that their glucose tolerance was altered in diabetic direction. The skin lesions usually follow cardiac decompensation, with or without oedema of the legs, or unilateral oedema of the leg due, for example, to venous thrombosis. The author suggests that the lesions are due to qualitatively or quantitatively altered reaction in diabetics. The patients may also have skeletal destruction in the feet. The initial lesion is dermatopathia diabetica (Melin) are also described. They consist of superficial ulcerations the size of corns or somewhat larger surrounded by a narrow zone of erythema.

Diabetic skin lesions have been studied at the Department of Medicine in Umeå for over a decade and in 1964 Melin (48) described small atrophic, well demarcated scars on the legs of diabetics. Melin's observations have been confirmed by a number of authors (3, 7, 14, 55) and these skin lesions are now usually called dermatopathia diabetica (7, 9, 10, 11, 15, 21, 22, 32, 38, 51, 64).

Another cutaneous lesion of the lower extremities apparently typical of diabetes has been observed at the Department of Medicine in Umeå. It consists of erythema of the skin of the leg or the foot. The centre of the area is often necrotic. To the author's knowledge they have hardly been described earlier. A close study has been made of

them including an attempt to identify possible precipitating factors.

Some patients with erythema and necrosis of the skin of the feet have been found also to have destruction of the bones of the feet. The simultaneous presence of skin lesions of the type described by Melin (48) has been recorded with special attention to the initial stages of such lesions which have received little attention (22). The present paper also describes a number of cases of erysipelas of the lower extremities.

Erythema in connection with diabetes has been observed earlier. Reddening of the face in diabetes was first described by von Norden and Isaak (59) who termed it rubeosis faciei. This phenomenon was studied in detail by Grieson and Wertheimer Kaplinski (74). Lundbeck (43) has described a condition which he calls rubeosis plantarum describing a total of distal gangrene of the lower extremities. Ipsen (31) mentions that in warm gangrene the border of the necrotic area may be reddened. Most of his patients were diabetics. "Ulcers of the ball of the foot, mal perforans" have long been known to occur in diabetics. They have been described by Marchal (45) in 1864. Pryce (63) in 1887 and others and have been assumed to be due to neuropathy (76). Erythema of the skin of the leg in connection with arterial ischaemia has been described (40) but information as to the possible concomitant presence of diabetes is lacking. Ischaemic ulcers of the legs (17, 18, 30) and so-called hypertensive ulcers of the legs (47) have also been described without mention of whether diabetes was present or not. Skeletal destruction in association with distal diabetic gangrene with ulceration has been described (40). Destruction of the bones of the foot not obviously connected with skin ulcerations was probably first

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Table 1 Data in the patients in groups A and B

N	Age (y)	Precipitating factor		Arterial insufficiency	Neuropathy	Swelling of erythematous zone	N swelling of erythematous zone	Diabetes not diagnosed
		Cardiac decompensation	Unilateral leg oedema					
Group A								
4	40-59	3	1	1			2	1
33	>59	1	1	7	18	21	12	16
Total	37	15	13	8	18	23	14	17
Group B								
7	40-59	1			1	3	4	
37	>59	19		14	18	16	21	10
Total	44	20		16	19	19	25	10

mentioned by Jordan in 1936 (33). The skeletal destruction may heal (49, 61, 62).

MATERIAL, DEFINITIONS AND METHODS

Cutaneous erythema of the leg, with or without necrosis, was observed in 94 patients. Among these patients there were 62 with known diabetes and 32 who were not recognized diabetics. Of the 94 patients, 79 were hospitalized in the Department of Medicine and 15 were outpatients. 14 of the latter were known diabetics. Of the 62 patients with known diabetes, 1 were on insulin, 40 on oral antidiabetic drugs and 1 was treated with diet alone.

The patients were collected over a period of little more than four years in the Department of Medicine, Umeå University, which serves a population of somewhat over 100,000 persons. Collection of the material was not entirely comprehensive. The described skin lesions, erythema or erythema and necrosis, were always manifest when the patient was admitted to the hospital and antibiotic therapy had not been given prior to the admission.

Known diabetes was recognized diabetes or "open diabetes" after previously recorded diagnoses of diabetes or to the presence of manifest, non-renal glucosuria at the time of examination. The parts described as reddened or erythematous were at least intensely red. Swelling refers to swelling so pronounced that finger pressure left a clear impression. Neuropathy refers to the absence of Achilles and patellar reflexes. Vibratory perception in the malleoli was always definitely impaired. By arterial insufficiency is meant the absence of pulses in both the *a. dorsalis pedis* and the *tibialis posterior*. These patients nearly always also lacked a pulse in the *a. poplitea*. In many cases pronounced oedema made it impossible to ascertain whether neuropathy or arterial insufficiency was present. In the tables these are tabulated as cases in which neuropathy or arterial insufficiency was absent. Skeletal destruction localized to the bones of the foot refers to the skeletal lesions

described in the literature (6, 13, 33, 36, 37, 46, 66), demonstrated roentgenologically. By erysipelas is meant skin lesions accompanied by reddening and heat as described in the literature (67) in patients with increased temperature and in whom there was an elevated antistreptolysin titre (AST) (34) during the period of observation.

Patients with erythema on the medial aspect of the leg, with or without ulceration, are not included in the material in view of the common occurrence of varicose lesions in this location.

All the patients on whom the above mentioned skin lesions were observed were entered in a register and the lesions were photographed in colour. The examination was performed with the patient in recumbent position.

Patients with the skin lesion listed who were not known diabetics were investigated by an oral glucose tolerance test in which 50 g glucose was administered. Blood glucose was determined by modification of the glucose oxidase method (28) with the patient in fasting state and then every half-hour for 4 hours following the administration of glucose. The blood glucose values in mg/100 ml were plotted on the ordinate of a coordinate system. Time in half-hour intervals was plotted on the abscissa. When the blood glucose values were connected 8 parallel trapezoids were obtained having the same height (=1 hour) and various lengths according to the individual blood glucose values. The area of each trapezoid was calculated as well as the combined area of the 8 trapezoids. The latter area represents a measure of the glucose tolerance.

The significance of the difference between means was calculated using Fischer's *t*-test. Fischer exact probability test and χ^2 -analysis were also used.

RESULTS

Group A

Erythema of a skin area at least the size of the palm of the hand on the anterior lateral or pos-

ration of open diabetes (%)

5-9	10-14	15-19	>19
5	1	2	
5	1		
8	5	3	4
10	7	3	6

terior aspect of the lower leg or reddening of the skin over most of the dorsum of the foot. 37 patients (16 men, 1 women) with an average age of 73 years (men 71 women 74). The 20 patients with known diabetes had had the disease for on an average 5.4 years (range 1-16) (Table I Figs. 1b and 2).

Thirteen patients had erythema only on the foot, 10 only on the leg, and 14 on both foot and leg. Fifteen patients had bilateral erythema of the lower extremities. In 7 of the patients the duration of the erythema could be determined and varied from 5 to 22 days. In these 7 patients the reddened area was swollen, the swelling had always preceded the erythema by from 3 days to an indefinite time. When the swelling receded the erythema always disappeared. Of the 15 patients in whom cardiac decompensation was stated to be precipitating factor, 11 had definite oedema of the lower leg.

Phlebography was carried out in 9 of the 13 patients with unilateral leg oedema and deep venous thrombosis demonstrated in 6 of them.

Six (16%) of the patients had reddening of the soles of the feet.

Eight patients also had small atrophic scars on the anterior aspect of the leg, of the type described by Mehn (48). Three of the patients with cardiac decompensation or unilateral leg oedema also had signs of arterial insufficiency. Two of the patients with unilateral leg oedema also had cardiac decompensation. In Table I they are included among patients with unilateral leg oedema. In one patient with recognized diabetes, widespread reddening and swelling of both feet occurred in connection with increased severity of chronic rheumatoid arthritis. In 6 of the patients no precipitating factor such as cardiac decompensation or unilateral leg oedema could be ascertained. Neither did these patients have signs of arterial insufficiency. Five of these 6 patients are known to have diabetes.

The frequency of known diabetes among patients in whom precipitating factors such as cardiac decompensation,

non, or lateral leg oedema or arterial insufficiency were recognized was 15/31. The difference between 5/6 and 15/31 is statistically significant ($p < 0.02$).

Gump B

Necrotic ulceration of an area of skin ranging in size from that of confetti to that of a child's palm with or without a crust surrounded by intense erythema. The ulcer was located on the dorsum, the lateral aspect or the sole of the foot or on the anterior, lateral or posterior aspect of the leg. 44 patients (16 men, 28 women) with an average age of 69 years (men 74 women 65). The 34 patients with known diabetes had had the disease for on an average 11 years (range 1-34) (Table I Figs. 1a and 3).

The erythematous area had always been many times larger than the area of necrosis. The necrosis involved all layers of the skin and was nearly always painless.

Of the 20 patients in whom cardiac decompensation was stated to be a precipitating factor, 12 had definite oedema of the lower leg. Only 14 of the 44 patients were able to give an account of how the necrosis began. In 11 it had begun as a large zone of erythema in which central necrosis gradually developed. In 3 it had begun as a small red spot.

The necrosis was located on the foot in 36 patients (the dorsum in 5, leg in 7, leg 11, in 1 the heel in 6, the lateral border in 3, an interdigital space in 1, the sole in the region of metatarsal I in 2, and the sole in the region of the head of metatarsal V in 1) on the lower leg in 16 and on both the foot and lower leg in 2. In the 16 patients with necrosis on the leg, the anterior, posterior and lateral aspects were affected in 3, 4 and 9 cases respectively. Seven of the patients with necrosis on the foot and 5 with necrosis on the lower leg had multiple necroses, the largest of which is given as the main site. Nineteen (43%) patients had reddening of the soles of the feet. Nine patients also had small atrophic scars on the anterior aspect of the leg, of the type described by Mehn (48).

In the cases in which it was possible to follow the clinical course the necroses usually healed, but 4 patients underwent amputation of the affected part. Seven patients died of the acute illness responsible for their admission, before the necroses had healed.

Eight of the patients with cardiac decompensation or unilateral leg oedema also had signs of arterial insufficiency. In 14 of the patients no precipitating factor such as cardiac decompensation or unilateral leg oedema could be identified. Neither did these patients have signs of arterial insufficiency. Thirteen of these 14 patients were known to have diabetes. The frequency of known diabetes among patients in whom precipitating factors such as cardiac decompensation, unilateral leg oedema or arterial insufficiency were recognized was 21/30. The difference between 13/14 and 21/30 is statistically significant ($p < 0.001$).

Table II Data on the patients in group C

N	Age (y)	Swelling of affected part	No swelling of affected part	Neuropathy	Duration of open diabetes (y)				
					0-4	5-9	10-14	15-19	>19
4	40-59	3	1	4		1	1		2
6	>59	3	3	4	1	1	2	1	1
Total	10	6	4	8	1	2	3	1	3

Among the patients in group B the frequency of known diabetes was 77.3% and among the patients in group A 54.1% ($p=0.017$) (Table I). In the former group the mean duration of diabetes was 11.0 years and in the latter 5.4 years. Calculation of the significance of the difference between the means for the two groups yielded $t=3.20$ d.f. 92 and $0.001 < p < 0.005$.

Group C

Destruction of the bones of the foot demonstrated roentgenographically in patients with cutaneous necrosis on the foot 10 patients (2 men 8 women) with a mean age of 63 years (Table II). All of these patients are also included in group B. All were known to have diabetes and the mean duration of the disease was 17.0 years (range 3-34).

Three of the patients had widespread destruction in several of the metatarsal bones especially in the metatarsophalangeal region, 3 had similar changes localized to the metatarsophalangeal region of digit V. 4 had destruction in the distal phalanx of digit I and 1 had destruction of the middle phalanx of digit II. One patient, a 65-year-old woman with diabetes of 22 years' duration who had multiple ulcers on one foot had destruction of most of the metatarsal bones, bones of the middle foot, the talus and calcaneus laterally and the distal end of the right tibia. She had skin necrosis/necroses over the site(s) of skeletal destruction. The necrotic area was always smaller than the region of skeletal destruction. The bone lesion was never exposed. In one patient the area of cutaneous necrosis was far removed from the region of skeletal destruction and in 3 the cutaneous necrosis was on the other foot.

Eight of the 10 patients had neuropathy; in the remaining 2 it could not be evaluated.

In a further 8 patients with cutaneous necroses on the foot X-ray revealed a normal bone structure and in the remaining 10 X-ray examination was not carried out. The latter 10 patients were among the first observed before the author was aware of the connection between the skin lesions and skeletal destruction. In one patient bone destruction was demonstrated first skin lesions later.

In the cases in which the skin over the site of skeletal destruction was intact, it was not possible to determine anamnestically whether skin lesions had earlier been present.

Group D

Erythema alone or erythema and necrosis recurring after healing 6 patients (2 men 4 women) with a mean age of 68 years (range 46-76). One of these patients is also included in group A and five in group B.

The recurrence in four patients consisted of erythema and in two of erythema and necrosis. Recurrence did not always involve the same site as the initially observed skin lesions. The initial lesions were confined to one leg, but in three cases recurrence involved both legs. In two of these recurrence was associated with cardiac decompensation and oedema of the legs. In one of the six cases recurrence was associated with local arterial insufficiency.

The average interval between the first observed skin lesions and the recorded recurrence was 1 1/2 years (9/10-2/10). It should be noted that the time of recurrence could not be determined with greater accuracy than to the nearest month. Five patients had neuropathy and four had arterial insufficiency. All were known diabetics with a mean disease duration of 11.2 years (range 3-24).

Group E

Encrusted ulcers the size of confetti or somewhat larger surrounded by a reddened border 8 patients (5 men 3 women) with a mean age of 61 years (Table III). The ulcers were located on the anterior aspect of the leg and involved only the superficial layers of the skin. The ulcers were multiple in 7 cases and arranged in a linear pattern or in groups (Fig. 4).

Seven of the patients were known diabetics with a mean disease duration of 12.4 years (range 1-29). The patient who was not a recognized diabetic had a clearly diabetic type of glucose tolerance test.

In the three cases in which it was possible to follow the clinical course the described lesions gradually developed into typical atrophic spots of the type described by Mehm. All had atrophic skin lesions of the Mehm type at the time of the first examination.

Group F

Patients who were not known to have diabetes and who had only erythema or erythema and necrosis:



Fig 1a. Female, 73 years. Diabetes of 13 years' duration. In association with cardiac decompensation and bilateral leg oedema, the patient developed erythema of both legs and feet and necrosis on the lateral aspect of the right leg.

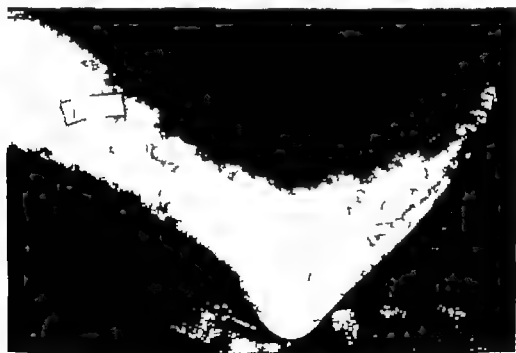


Fig 1b. The same patient 10 months later when in association with recurrence of cardiac decompensation and leg oedema she had developed erythema of the distal parts of the legs and the feet. The areas of skin necrosis had now healed.



Fig. 2. Male, 71 years. Diabetic oral glucose tolerance curve. Swelling of the right leg and foot. (Thrombosis verified by phlebography.) Erythema of the dorsum of the right foot.

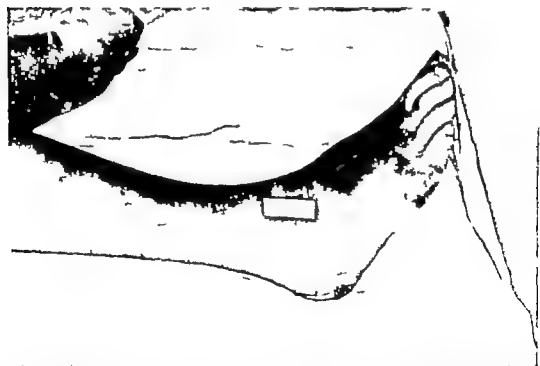


Fig. 3. Male, 82 years. Diabetes of 3 years duration. On the lateral border of the right foot, a small area of necrosis accompanied by widespread erythema of the entire dorsum of the foot and the distal part of the leg.

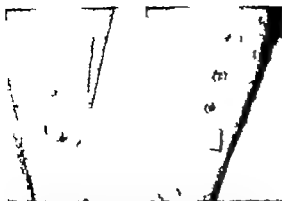


Fig. 4. Male, 69 years. Diabetes of 6 years duration. Multiple encrusted ulcers on the anterior aspects of both legs 7-8 months later typical atrophic scars.

28 patients (16 men 12 women) with a mean age of 71 years (men 77 women 71).

These patients underwent an oral glucose tolerance test and the results were compared with those of 35 controls. Of these 28 patients 17 are also included in group A, 10 in group B and 1 in group E.

Of the 35 controls 19 were men and 16 women with mean age of 73 years (men 73 women 71). All were hospitalized with diagnoses as shallow as possible to those of the patients with skin lesions on the lower extremities not known to have diabetes. The controls were otherwise unselected and several of them proved to have clearly diabetic glucose tolerance curves.

The oral glucose tolerance tests were always performed when the patient acute disease if any was in remission and the patient was afebrile. The results were treated as described under Material definitions and methods. The glucose tolerance of the 28 patients not known to have diabetes was impaired in comparison

with that of the 35 controls. The mean values for glucose tolerance calculated in the first described manner were 569 and 456 respectively. The average mean errors were 21.3 and 11.5 respectively. Calculation of the significance of the difference between the means for the two groups yielded $t=3.93$ d.f. = 61 and $P<0.001$. Comparison of the values for the controls with

those of the 17 patients with erythema alone and of the 11 with erythema and necrosis yielded $t=3.25$ d.f. = 50 $0.001<P<0.005$ and $t=67$ d.f. = 43 $0.01<P<0.02$ respectively.

Group G

Erythema 5 patients (one 54-year-old man, 4 women with a mean age of 58.3 years). One woman aged 67 was known to have had diabetes for four years. The other patients were not known diabetics.

All had initially an oral temperature of $39.0-39.9^{\circ}$ and an elevated AST (400-1600 U). All had superficial ulcers, deep ulcers or fissures of the skin adjacent to the reddened area which in three cases involved the foot and leg. In two cases only the leg. In four patients the affected leg was markedly swollen. None had cardiac decompensation.

The glucose tolerance tests revealed no significant difference between these patients and the controls ($t=1.38$ d.f. = 37 and $0.10<P<0.20$).

DISCUSSION

The skin lesions described characterized by erythema or erythema and necrosis are localized to the distal part of the lower extremities. They occur in persons especially in the elderly with recognized diabetes, but are also seen in elderly persons not known to have diabetes. However, comparison of the glucose tolerance test values for a group of the latter type of patients with those of a control group showed a clear alteration in the diabetic direction. The difference was statistically highly significant. In other words the skin lesions are closely connected with diabetes.

The skin lesions are usually precipitated by cardiac decompensation with or without oedema of the legs or by unilateral leg oedema due for example to venous thrombosis (Table 1). They may possibly be precipitated by arterial insufficiency.

Table III. Data on the patients in group E

N	Age (y)	Neuropathy	Swelling of affected part	No swelling of affected part	Diabetes not diagnosed	Duration of open diabetes (y)			
						0-4	5-9	10-14	15-19
2	20-39	2		2					
1	40-59			1	1				
5	>59	4	1	4		1	3		1
Total 8		6	1	7	1	1	3		1

Entmacher et al (70) state that cardiac disease is 2.0 times as frequent a cause of death among diabetic men as among men in general. The corresponding figure for women is given as 3.0. Of the precipitating factors mentioned above cardiac decompensation would therefore be expected to be more common among diabetics than in the population at large. Whether unilateral leg oedema is more common among diabetics than in the general population is not known. In Sweden the frequencies of recognized diabetes in the age groups 60-69 and 70-79 respectively are 6% and 4.3% for men and 3.3% and 4.2% for women (54).

Since the factors precipitating the above mentioned skin lesions can be assumed to be 2.0 and 3.2 times more common among diabetic men and women respectively than in the population at large, the author's finding that about 53% of the men and about 76% of the women with such skin lesions have open diabetes suggests that the development of such lesions reflects a quantitatively or qualitatively altered reaction to the precipitating factors on the part of diabetics. Whether this altered reaction is due to an altered metabolism, immunoreaction included, in the cells of the diabetic tissue or whether it is due to an altered blood supply of this tissue which may cause decreased transport of oxygen to the tissue or of metabolites from the tissue or both cannot be decided.

Among the patients with erythema and necrosis the frequency of known diabetes was 77.3% and among those with erythema alone 54.1%. The former group differed from the latter in having a longer duration of diabetes (11.0 years compared to 5.4 years). In both cases the difference was statistically significant. Erythema and necrosis must be regarded as a more powerful reaction than erythema alone. Thus open diabetes and a long duration of the disease apparently predispose to a more powerful reaction to precipitating factors.

It is striking, in regard to both erythema (group A) and erythema with necrosis (group B) (Table I) that precipitating factors in the form of cardiac decompensation, unilateral leg oedema and possibly arterial insufficiency were statistically significantly more common among patients not known to be diabetics than among those with open diabetes. This may mean that the assumed alteration in mode of reaction is less pronounced in patients of the former type and that in these patients the skin

lesions usually develop only in response to a more powerful precipitating factor.

The author has not found any connection between the skin lesions discussed and arterial hypertension.

The initial development of erythema alone was followed in a number of patients with oedematous swelling of the part in question. It was obvious that the swelling always preceded the development of erythema and that the latter receded when the swelling subsided (compare the description of group A). The skin lesions were usually reversible. Even necroses healed in over half of the patients. Exact figures are lacking since some of the patients died during the period of observation, in others the affected extremity was amputated and for some patients complete clinical follow-up was impossible.

It is characteristic that the skin lesions may recur after once having healed, may be at a site other than that at which they were first observed. It is striking that all the patients with such recurrences had known diabetes of long duration (compare the description of group D).

X-rays of the feet of patients with skin lesions often revealed skeletal destruction (group C, Table II). These patients were all known diabetics with a long mean duration of the disease. The skin over the sites of the bone lesions may be either intact or altered.

There is probably no clear distinction between cutaneous erythema and necrosis and what is usually termed distal diabetic gangrene localized, for example to the toes or heel. Of the 44 patients in group B three had obvious distal black diabetic gangrene involving the heel or the entire circumference of one or more toes. It has long been known that distal gangrene of the lower extremities is common in diabetics (5).

Diabetics have often been reported to have good pulses in spite of the presence of gangrene of the toes, in comparison with non-diabetics with atherosclerotic vascular foot lesions which usually have intermittent claudication and absence of pulses in the a. poplitea and the a. tibialis posterior (50). In only a few of the patients in the present study with erythema or erythema and necrosis localized to the distal part of the lower extremities were the peripheral pulses absent (Table I). In most patients the skin of the lower leg was warm and there were good pulses in the

a. dorsalis pedis the a. tibialis posterior and the a. poplitea. Only a few of those who lacked peripheral pulses had cold extremities or intermittent claudication. The clinical significance of absent pulses or demonstrable arterial occlusion is however not clear (40). Widmer et al (68) in an investigation of 6400 industrial workers performed angiography in 83 persons and demonstrated 99 peripheral arterial occlusions in 75 of them. Two-thirds of these persons were entirely free of symptoms.

The investigations of the circulation in the lower limbs of diabetics (1, 35-53, 58) fail to support the assumption that arteriosclerotic lesions and lesions in the smaller vessels have any obvious effect on the blood supply to the legs of diabetics with disease of long duration even under conditions of muscular work. It appears to be an open question whether the blood supply to the feet and toes is impaired in diabetics. In patients with distal gangrene in association with occlusion of the chief arteries of the leg, ¹²⁵I-xenon clearance studies have shown that the blood flow through the skin on the dorsum of the foot is diminished (39). Data on diabetics are lacking.

Some earlier authors (4, 16, 42) consider that gangrene of the distal extremities in diabetics is due to arteriosclerosis. In discussing the incidence of arteriosclerosis a distinction should be made between calcification of the media (Mönckeberg) and lesions of the intima (atherosclerosis). The former does not usually affect the arterial lumen (41) and is generally accepted as being more frequent among diabetics but many authors (29, 44, 65) have regarded it as lacking in clinical significance. On the other hand it is controversial whether intimal lesions are more common among diabetics than among non-diabetics (8, 25, 57).

Skeletal destruction in the feet of diabetics has been attributed to neuropathy by a number of authors (1, 2, 3, 27, 33, 36, 37, 46, 56, 66). Diabetic neuropathy is especially apt to involve sensory fibres (19). Although patients with skeletal destruction always have neuropathy (6) there is no convincing evidence that the latter is the cause of the skeletal lesions (60, 61).

As pointed out above there is no clear distinction between the skin lesions in diabetics characterized by cutaneous erythema and necrosis and distal diabetic gangrene. The author has further ascertained that the skin lesions are usually pro-

duced by cardiac decompensation, with or without leg oedema or by unilateral leg oedema due for example to venous thrombosis, and has suggested that their development depends on a quantitatively or qualitatively altered mode of reaction in diabetics as opposed to non-diabetics. The hypotheses regarding the cause of distal diabetic gangrene presented in the literature do not appear to be based on convincing evidence (13).

Superficial encrusted ulcers the size of confetti or somewhat larger surrounded by reddened margins were observed in 8 of the present patients. The ulcers were localized to the anterior aspect of the leg were multiple and arranged in a linear pattern or more irregularly in groups. All patients with such lesions were diabetics. In the three patients in whom the clinical course of the lesions could be followed they developed into skin lesions of the type described by Mehn (48).

Both the skin lesions described in the present study and by Mehn occur almost exclusively in diabetics and are virtually confined to the leg. In the cases in which the initial stage of the skin lesions described by Mehn were observed they consisted of confetti-sized ulcers surrounded by a marginal zone of erythema. The lesions described in the present study (Table I) also include erythema. A further common feature is that the lesions may heal and then recur. It should be added that the lesions described by Mehn may also be observed in younger persons with long-standing diabetes. Thus there are similarities between the two types of lesions.

The present author has suggested that the skin lesions described are due to qualitative or quantitative differences in mode of reaction in diabetics as opposed to non-diabetics. The fact that these lesions and those described by Mehn are confined to the lower leg may be due at least in part to the fact that the proposed alteration is confined to or more pronounced in the lower extremities. This assumption is supported by the following observations.

In one of the diabetic patients in group E (Table III) the author elicited erythema of the skin on the back of the hand and on the anterior aspect of the leg by means of a pencil of solid carbon dioxide 8 mm in diameter. On the leg the erythema developed into an ulcer surrounded by a reddened zone (Fig. 5a). The ulcer eventually gave rise to an atrophic scar of the type described by Mehn

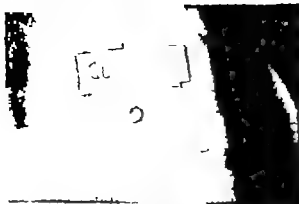


Fig 5a Male 38 years. Diabetes of 1 years duration. Ulceration and erythema of the anterior aspect of the right leg following traumatization with pencil of solid carbon dioxide 6 days previously



Fig 5b The same patient 8 months later. The final result of traumatization is the second spot distal to the figure 5a.

(Fig. 5b) The patient already had a number of such scars on the anterior aspect of the leg. On the back of the hand the erythematous reaction disappeared without a trace. In a similar experiment in a control person erythema disappeared without sequelae on both the leg and the hand.

The skin necroses on the legs and feet described in the present paper are always surrounded by a reddened zone. In the author's experience distal gangrene of the lower extremities in diabetes is also characterized by the presence of such a zone. The author has observed one patient, an 81 year-old woman with diabetes of long duration, who developed acute arterial circulatory insufficiency in the distal part of the right arm with slight swelling of the lower arm and a threat of gangrene of the fingers. The cause was probably arterial emboli. There was never any zone of erythema adjacent to the region threatened by gangrene.

On the basis of appearance alone it may be difficult to distinguish the skin lesions described here—cutaneous erythema and erythema accompanied by necrosis—from erysipelas of the leg. However the patients with the described skin lesions usually are afebrile and have neither increased ESR nor leucocytosis. Such findings when present, have always been attributable to another acute disease. In doubtful cases AST has been determined; the values have always been normal. In the course of this investigation 5 patients were encountered who fulfilled the earlier criteria for the diagnosis of erysipelas (group G). Among those in this group who were not known

diabetics the oral glucose tolerance test revealed no statistically certain difference from the controls.

At present clinical observations on lesions in the lower extremities of elderly diabetics can be summarized under eight fairly well defined categories. 1) Distal gangrene. In the author's experience characterized by a marginal zone of erythema. Aetiology unclear. 2) The skin lesions described by the author. Localized to the lower leg or foot and characterized by erythema and/or necrosis. They are usually precipitated by cardiac decompensation, with or without oedema of the leg or by unilateral leg oedema due for example to venous thrombosis. Both erythema and necrosis usually heal but the lesions may recur. The distinction from distal diabetic gangrene is not a sharp one. 3) Rubecosis plantarum (43). 4) Dermopathia diabetica (48). The skin lesions begin as superficial ulcers the size of confetti or slightly larger surrounded by a reddened margin. They develop into atrophic scars which also show increased vascularization. The lesions may heal and later recur. 5) Necrobiosis lipodica diabetorum. Rare especially in elderly diabetics (57). 6) Peripheral neuropathy. Aetiology unknown. 7) Arthropathia diabetica (Charcot joints). The patients always have peripheral neuropathy and the joint lesions are generally regarded as secondary to the neuropathy. 8) Skeletal destruction localized to the bones of the feet. The skin over the affected part is often intact. The destructions may heal. These patients always have peripheral neuropathy.

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CONGENITAL ABSENCE OF PORTAL VENOUS SYSTEM IN A 50-YEAR-OLD WOMAN

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Abstract. A 50-year-old woman is reported who had no portal vein at autopsy but otherwise the liver histology and function appeared unremarkable. Retrospectively it could be assumed that she had in fact had episodes of portal encephalopathy.

In 1793 Abernethy (1) described the autopsy findings of a girl with 'vena portarum terminating in the inferior caval vein'. Kjellman (3) in 1833 reported a similar anomaly in a 13-year-old girl who died from injury to the head and in 1934 Hellweg (2) described the autopsy findings of a 5½-month-old girl with congenital absence of the terminal portion of the portal vein.

The present paper describes the clinical history and necropsy findings in a 50-year-old woman with a similar agenesis of the portal venous system and discusses the possible effects of this anomaly on her cerebral function.

CASE REPORT

The patient was considered to be in good health, including a normal pregnancy when she was 25 years old. She had an appendectomy when she was 39 and subsequent intestinal obstruction caused by peritoneal adhesion when she was 42 years old. Starting at about 40 years of age she noticed a progressive tiredness and dyspnoea.

At examination at the age of 42 suggested an atrial septal defect. But at thoracotomy which was performed actually because of pericardial tamponade produced during attempted left-sided angiocardiology no septal defect was demonstrable. Pulmonary hypertension was demonstrated at cardiac catheterization and lung biopsy was performed. This displayed diffuse, almost completely interstitial fibrosis. It was concluded that the patient probably had an early Haemman-Rich syndrome. Because of slight hyperbilirubinaemia (1.2 mg/100 ml) liver biopsy was performed and was considered normal. There was no history of venous thrombosis, but since pulmonary embolism could not be de-

finitely excluded she was treated with dicoumarol during the subsequent 5 years. For the remainder of her life she was incapacitated by dyspnoea, and X-ray examination of the lungs showed progressive widening of the pulmonary trunk and main branches. Liver function tests were performed on several occasions and showed maximum bilirubin of 1.1 mg/100 ml and normal levels of transaminases, alkaline phosphatases and thy-mol turbidity.

Beginning at the age of 44 the patient was repeatedly admitted to the hospital because of drowsiness, tremor and unsteady gait. Since urinary analysis showed high concentrations of barbiturate and meprobamat and the symptoms disappeared after hospitalization, it was concluded that they were attributable to drug abuse.

The patient constantly complained of tiredness and weakness in the extremities and when she was 45 she was again hospitalized. Her symptoms, which disappeared after day were attributed to overdosage of diazepam. The muscular weakness continued to be prominent problem and she complained that on occasion she was so tired that she 'fell out of the chair when sitting'. She denied further consumption of sedatives, and her only known medications were digitalis, diuretics, potassium and dicoumarol. At the age of 46 she was noted to have hand tremor, vertigo in the upright position and slow speech and was described as very dull. Routine neurological examination was other-wise unremarkable with the exception of bilateral weakness in arms and legs. An EEG showed slow waves bilaterally without focal changes. A second EEG when she was 47 displayed progressive abnormality but still without difference between the two sides. There was practically no activity in the α -domain.

Hypercapnia, hypokalaemia and hypothyroidism were excluded causes of her neurological symptom. Her vertigo became more prominent. It was described as non-rotatory and was not accompanied by any hearing loss, difficulties of vision or paresis. Her speech was now described as slurred. Intermittently there was also inversion of the sleep rhythm. However it was repeatedly noted that there was no disorientation. No further deterioration was noted until at the age of 50 when she was admitted to the hospital with pulmonary oedema which did not respond to treatment.



Fig. 1 Microphotograph of liver showing absence of portal triads and abundance of hepatic arteries. Elastin + van Gieson. (a) $\times 12$, (b) $\times 216$.

At autopsy there was a marked hypertrophy and distension of the right atrium. The right atrium was filled with mural thrombi. There was no septal defect. The whole pulmonary tree was greatly dilated with largest circumference of the pulmonary trunk of 22 cm. The wall of the pulmonary arteries was thin and friable. There were several pencil-sized endotheleum-adherent pulmonary emboli, several pulmonary infarctions and pulmonary oedema. Femoral artery, and caval veins were normal.

The superior mesenteric and the splenic veins united normally but instead of extending into the liver the portal vein terminated in the superior caval vein just above the renal veins. The hepatic veins, which appeared small terminated normally in the inferior caval vein. The coeliac axis branched normally to the hepatic artery which was of about twice the normal size. The liver weighed 1100 g and had a smooth surface and a reddish edge. The cut surface showed exaggerated lobular architecture. At microscopic examination no intrahepatic branches of the portal veins were recognized in the portal areas which usually contained several arterial vessels (Fig. 1 and b).

The central nervous system was unremarkable macroscopically but at the right posterior cerebellar fossa, about 5 cm to the right of the falx cerebelli there was a meningioma, about 1 mm thick and about 1.5 cm in diameter. No further anomalies were detected on macroscopic examination.

DISCUSSION

Portal encephalopathy has only occasionally been reported in patients without liver disease but with appreciable portal systemic communications due to extrahepatic obstruction of the portal vein (5). It is therefore of interest to discuss whether the patient reported here suffered from portal encephalopathy during her last years.

Her neuropsychiatric symptoms (tiredness, hand tremor, drowsiness, inversion of sleep rhythm, slow and slurred speech) are all commonly encountered in portal encephalopathy (4). The vertigo which was a prominent symptom is not a characteristic symptom of portal encephalopathy but may well have been caused by her meningioma. However, the latter could not reasonably have caused the other neuropsychiatric symptoms. Though there is no specific EEG pattern for portal encephalopathy, other causes of the patient's EEG pattern such as hypoglycaemia, uraemia, hypercapnia and grave electrolyte disturbances could be excluded. Overdosage of sedatives could theoretically have contributed to the clinical picture at the time of hospitalization and probably did so on the first admissions for tiredness and drowsiness but later sedatives were not prescribed. Furthermore, the EEGs were registered after several days of hospitalization when she did not receive any sedatives. Apart from a slightly elevated serum bilirubin concentration there were no biochemical criteria for hepatic dysfunction, and the liver morphology was normal except for the absence of portal veins and the abundance of hepatic arteries. It thus seems probable that the neuropsychiatric symptoms in this woman were caused by the portal blood bypassing a liver without hepatocellular failure.

Since these symptoms did not appear until after her cardiopulmonary function had deteriorated because of pulmonary embolism, pulmonary hypertension and lung fibrosis, it seems likely that this impairment in some way was of importance for the development of the portal encephalopathy.

Hypoxia, CO_2 retention, alkalosis and hypokalaemia were all excluded as contributory factors. However, it cannot be ruled out that a progressively increasing right heart failure with congestive liver damage could have been of importance for the development of the portal encephalopathy.

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HYPOGLYCEMIA CAUSED BY AN INTRATHORACIC TUMOUR

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Abstract. A patient with a large intrathoracic tumour associated with hypoglycemic attacks and whose fasting glucose levels were frequently below 30 mg/100 ml is described. The immunoreactive insulin (IRI) level during the hypoglycemic attacks and fasting was less than 5 μ U/ml. The oral glucose tolerance test was normal. Glucagon gave a subnormal increase in blood glucose and no increase in serum IRI levels. Diazoxide did not reduce the frequency of attacks. The hypoglycemic attacks subsided after removal of a 5 kg cell-rich mesenchymal tumour which was probably neurofibroma. The IRI concentration in the primary tumour extract was 0.05% of that found in extract from normal pancreatic tissue and only 0.0005-0.1% of that reported for insulinomas. On Sephadex LH gel filtration the low IRI activity was not eluted as insulin or proinsulin. All findings support the view that the hypoglycemia was not caused by insulin production by the tumour. Other possible causes are discussed.

Insulinoma induces hypoglycemia by causing high serum insulin levels through insulin release. The insulin content in tumour cells varies widely and is probably dependent upon the storage capacity of the cells for insulin (6).

Hypoglycemia may occasionally be associated with different extrapancreatic tumours (1-3, 4, 5, 10, 11, 13, 14, 15, 16, 17, 19, 21), e.g. hepatoma, suprarenal tumours, tumours originated from appendix or ovaries, different epithelial tumours and finally tumours of mesenchymal origin as first described by Doege (7) and Potter (18). The mechanism whereby such extrapancreatic tumours cause hypoglycemia, and particularly whether such tumours produce insulin is still under dispute.

In the present work a patient with a large intrathoracic tumour, probably of the Doege and Potter type, was investigated. Evidence is presented which suggests that insulin production by the tumour did not cause the hypoglycemia. Other explanations are discussed.

CASE REPORT

The patient, a woman born in 1926, had previously been in good health. In 1967 she developed marked hypertrophic osteoarthropathy. In 1969 butterfly skinburns developed on her face and the diagnosis of LED was made in spite of negative laboratory test for this disease, however treatment with prednisone and azathioprine was initiated.

During 1971 she complained of dyspnea during exertion and in Jan. 1972 also when resting (Figs 1 & 2) and showed the development of an intrathoracic tumour which, in retrospect, could have been noticed on X-rays already in 1959. The tumour growth was slow until 1970 and had been falsely diagnosed as an elevation of the right diaphragm. In Jan. 1972 the right lung was totally collapsed by a huge tumour which entirely filled the right side of the chest causing a marked displacement of the mediastinum. The tumour was shown by angiography to be supplied by bronchial arteries.

Blood glucose homeostasis

In June 1971 the patient experienced slight fasting attacks, transient hemiplegia and dysphasia lasting for hours. She felt that these attacks were relieved by taking prednisone tablets and the dosage of prednisone was increased from 15 mg each alternate day to 20 mg daily. No further attack occurred until Jan. 1972, when admission to hospital became necessary due to her experiencing recurrent fainting attacks and a diagnosis of hypoglycemia was then made.

Fig. 3 shows that hypoglycemia frequently recurred in the fasting state and rarely during the day. The oral glucose tolerance test (1 g glucose/kg b.wt.) was normal.

The serum immunoreactive insulin (IRI) levels during hypoglycemic attacks and during fasting determined on nine different occasions were all less than 5 μ U/ml serum. Glucagon (1 mg) gave no insulin release and only slight increase in the blood glucose levels (Fig. 3). Diazoxide 100 mg twice daily had no influence on the attacks or on the blood sugar levels. No antibodies to insulin were found in serum from the patient (8).

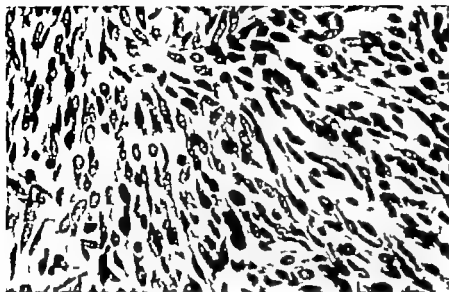


Fig. 4 Histology of the intrathoracic tumour consisting of cell-rich tissue of spindle cells running in whorls suggesting neurofibroma (magnification, $\times 256$).

that the small amount of insulin seemingly detected in the primary lung tumour extract had different properties. The substance might therefore not have been insulin and one could speculate as to whether other components were present. These could have possessed the same antigenic determinants as insulin; they might be modified insulin or insulin attached to some substance causing different chromatography characteristics.

Previous reports have given widely different results as to insulin content in similar tumours. High insulin-like activity has frequently been measured by bioassays (1, 2, 3, 4, 11). The more specific immunoassays have usually shown absence or small amounts of insulin similar to our findings (1, 4, 5, 11, 13). The extraction methods differed, however, and it is suggested here that substances other than insulin which have the same immunoreactivity could have been extracted in variable amounts. Volpe et al. (1) suggested a similar explanation, although tumour extracts were not examined.

The low preoperative insulin levels in blood even after β -cell stimulation are remarkable and similar to those found in other studies (4, 5, 11, 12). The prolonged absence of stimulation prior to the test is not a likely explanation, because the patient's blood glucose increased normally after meals (Fig. 2). This supports the view that such tumours, apart from being large glucose consumers (4, 5, 11, 12), also affect glucose regulating mechanisms (4, 5, 10, 11). The low insulin levels also

argue against a β -cell stimulator being secreted by the tumour.

Another possible mechanism of hypoglycaemia is increased glucose consumption by such large tumours. There is also evidence of ineffective glucose utilization in the glycolytic pathway (4, 5, 11). Decreased glucose output from the liver as demonstrated by the glucagon test, may also have contributed to hypoglycaemia. For a while prediabetic counteracted hypoglycaemic attacks in our patient, probably because of its diabetogenic effect.

An association with other endocrinopathies in the Doege and Potter syndrome has been reported; thus thyrotoxicosis has been found in 10% of cases (11). The non-functioning thyroid adenoma in our patient may be accidental.

The only effective treatment of the Doege and Potter syndrome has been found to be surgical removal of the tumour (11). In spite of the large size of such tumours, there is slight tendency to metastasis. However, recrudescence of the tumour may occur many years later, as reported in the Doege and Potter case.

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Announcements

The postgraduate course and workshop Progress in fibrinolysis organized by European Thrombosis Research Organization (ETRO) and Fondazione Giovanni Lorenzini will be held in Milan Italy March 6-8 1975

Fees Postgraduate course US\$ 50 Postgraduate course and workshop US\$ 100

Registration and information before Jan 31 1975 to: Fondazione Giovanni Lorenzini Via G. Lorenzini 7 (ex Via Brembo 65) 20139 Milan, Italy

The third European Conference of Internal Medicine organized by the European Association of Internal Medicine (A.E.M.I.E.) presided over by Professor E. F. Pfeiffer Ulm University will be held in the Hague The Netherlands May 1-3 1975

Topics Tropical diseases in Europe Thrombosis in internal medicine (new concepts in pathogenesis and therapy) General problems of internal medicine

Applications The Secretariat of the A.E.M.I.E. Dr J. Dagnelle rue Corsai 6c B-5861 Cortil-Noir mont, Belgium

International Conference on Prostaglandins will be held in Florence Italy May 26-30 1975

Co-chairmen Prof. B. Samuelsson and Prof. Paoletti.

Information The Secretariat of the International Conference on Prostaglandins Dr G. C. Folco Institute of Pharmacology and Pharmacognosy University of Milan Via Andrea del Sarto 21 20129 Milan, Italy

The fourth biennial course on current methods of immunologic research and diagnosis will be offered by The Center for Immunology of the State University of New York at Buffalo in July 1975

Deadline for applications March 31 1975

Further information James F. Mohn, MD Director The Center for Immunology State University of New York at Buffalo Buffalo New York 14214 USA

Digestion of milk A prize of Sfr. 15 000 will be awarded in Sept. 1975 by the Central Union of Swiss Milk Producers, Berne to a scientist (chemist, physician, biologist, who have worked along this field) from the following countries: Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Czechoslovakia, Denmark, Finland, France, India, Ireland, Israel, Italy, Japan, Kenya, Luxembourg, The Netherlands, New Zealand, Norway, Poland, South Africa, Spain, Sweden, Switzerland, United Kingdom, USSR, West Germany.

Applications Professor M. Demole, Unité de Diététique Hôpital Cantonal CH 111 Genève 4, Switzerland until Jan 31 1975. Applications should be accompanied by 3 copies of a) curriculum vitae b) list of works c) reprints of or 3 papers on the theme of the prize, published in the last 5 years (no typewritten papers). These documents should be written in English, French or German or should be accompanied by a translation into one of these 3 languages (will not be returned to the authors).

EDITORIAL

THE PHILADELPHIA CHROMOSOME AND CHRONIC MYELOCYTIC LEUKEMIA (CML) —STILL A COMPLEX RELATIONSHIP?

Except for a few reports of the presence of the Philadelphia (Ph⁺) chromosome in rare cases of non-CML myeloproliferative diseases (18) it still holds true that the Ph chromosome is exclusively associated with CML. But just does this relationship mean in terms of etiology and pathogenesis of the disease? Although these questions have so far not been sufficiently answered, recent research has thrown some more light on the Philadelphia problem.

Who has the Ph chromosome?

About 85% of the patients with CML have this abnormal chromosome in myelopoietic, thrombopoietic and erythropoietic precursor cells (17). A small number of patients are monoclonal for Ph⁺ positive and Ph⁺ negative cells, and the remaining 10-15% lack the abnormality (17).

The Ph chromosome does not generally disappear from the bone marrow during conventional treatment with busulfan (16). However, intensive treatment leading to hypoplasia of the bone marrow may eradicate the Ph⁺ positive cell population temporarily (2, 4). Such "over-treatment" has induced long-term remissions in single patients, but has so far been considered more risky than beneficial (6). However, the access to new cytotoxic drugs may change this view and the goal may become to eradicate the Ph⁺ positive cell population.

Which chromosome is affected?

For several years after the discovery of the Ph chromosome (14) it was not possible to distinguish between the two 22 chromosomes, i.e. members 21 and 22, and it was generally believed that the Ph⁺ chromosome was a number 21 chromosome. Thus, it was assumed that the Ph abnormality and the Q trisomy observed in Down's syndrome, condition with well known high risk for leukemia development, affected the same chromosome pair. In 1970 it became clear using the Q-banding technique that the Ph chromosome and the extra chromosome in Down's syndrome were not the same (1). The Ph⁺ chromosome was assigned to number 22 chromosome although the normal chromosome 22 will now with this designation be slightly longer than 21.

Up till most recently the Ph chromosome was regarded as a deletion of the long arm of chromosome 22, although it was suspected for long time that it might be the result of translocation. In 1973 it was demonstrated (15) that it is in fact the result of translocation between chromosomes 9 and 22. The missing part of the long arm of the Ph⁺ was found to be located

on the long arm of one of the number 9 chromosomes (15). This finding has rapidly been confirmed by others (8). As shown in this number of the *Acta Medica Scandinavica* (8) Ph⁺ negative patients with CML do not show the additional chromosome material on the long arm of chromosome 9 (or on other chromosomes) which provides further evidence that the extra material on chromosome 9 in Ph⁺ positive patients is translocated from 22. It was initially believed that this translocation was the only one associated with Ph⁺ positive CML. However, most recently it was found in three patients that the fragment from chromosome 22 could be translocated to other chromosomes i.e. chromosomes 4, 19 and the homologous 22 (5, 10, 12) without apparent differences in the clinical hematological pattern or course of the disease (8, 10). In fourth patient the missing chromosomal fragment on 22 was not found on any other chromosome (13). However, translocated fragment may have been undetected for technical reasons and it is not clear that this case is an example of a true deletion. Thus, it appears that the Ph⁺ chromosome is most frequently the result of translocation between 9 and 22, but may also rarely occur as result of translocation between 22 and other chromosomes (Fig. 1).

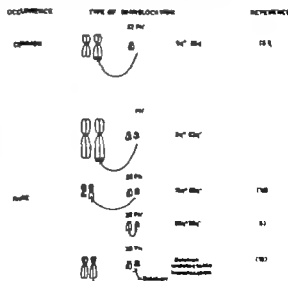


Fig. 1 Variant translocations in CML.

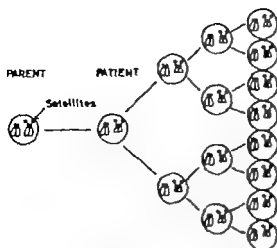


Fig 2 Clonal development of CML. Normal chromosomes 22 in parent cells. Ph acquired through translocation in one of the patient's stem cells committed for myeloid, erythroid and thrombopoietic cell differentiation. After multiplication Ph only in the satellite carrying chromosome 22 of leukemic cells.

Is the Ph chromosome acquired? If so in which chromosome 22—the one from the father or the one from the mother

It is well known that the Ph abnormality is acquired since it does not exist in myelopoietic cells in neither an identical twin (11) nor in parent of Ph positive CML patients (7-9). Furthermore, it can be acquired from either the maternal or paternal chromosome 22 (9). This could be shown using fluorescent satellites on chromosomes 22 as markers (2).

Does the abnormal cell population develop from a mutation in single cell or from simultaneous mutations in many cells

During the satellite study mentioned above it was found that the Ph abnormality was always found in the satellite marked chromosome 22 (Fig 2). In one patient the Ph chromosome was found in all metaphases in the satellite marked 22 received from the mother and in the other from the father strongly supporting the view that the Ph positive cell population has developed from one single cell (clonal origin). The only argument against such a view would be an inherited predisposition as the respective parental chromosomes for development of the Ph⁺ abnormality. Although unlikely such a predisposition could result in either a multicellular or unicellular (clonal) development. There is further evidence in favour of clonal origin, i.e. the fact that granulocytes from females with

CML and heterozygosity for glucose-6-phosphate dehydrogenase variants possess only one enzyme variant (3).

Unresolved problems?

There are many more problems to be resolved about CML and the Ph chromosome, i.e. how is the translocation induced? What are the etiologic agents inducing it? Is it a virus? Is it a different virus for different translocations? Furthermore and most important—is the Ph⁺ chromosome only the result of the disease or is the translocation the first step in its development? Hopefully these and many more questions about the Ph and CML, which are of importance for the understanding of the disease and thus ultimately for the treatment of the patient, will be resolved in the future.

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INVOLVEMENT OF CHROMOSOMES 8 9 19 AND 22 IN PH⁺ POSITIVE AND PH⁺ NEGATIVE CHRONIC MYELOCYTIC LEUKEMIA IN THE CHRONIC OR BLASTIC STAGE

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Abstract Chromosome 9 had additional chromosomal material at the end of the long arm in 15 of 18 patients with Ph⁺ positive CML. The remaining patient had a normal chromosome 9 but additional material on 19. The amount of additional material was approximately the same as that lacking on chromosome 22. Translocations between 9 and 22, and 19 and 22, respectively are therefore suggested. One patient with Ph⁺ negative CML had no detectable additional chromosomal material on any chromosome including chromosome 9 which gives further support to the view that there is indeed a translocation in Ph⁺ positive patients. Extra chromosomes appeared in two of three Ph⁺ positive patients in the blastic stage. Both patients had extra number 8 chromosomes and double Ph⁺ and one patient had further extra chromosomes, i.e. 7 11 12, 16, 17 and 19.

It has recently been shown using the Q-banding technique (6) that one of the number 9 chromosomes has additional chromosomal material on its long arm in chronic myelocytic leukemia (CML) (20). The amount of additional material has in most cases been approximately the same as that lacking on the Ph chromosome previously identified as number 22 (3). It has therefore been suggested that there might be a translocation between chromosomes 9 and 22 in CML. Only four patients with Ph⁺ positive CML and normal chromosomes 9 have so far been described. However additional chromosome material was found in three of these patients on other chromosomes (9 12, 16).

In the present work we also report one patient with Ph⁺ negative CML, in whom there was no additional

chromosome material on chromosome 9 or on any other chromosome—a further proof of the view that there is in fact a translocation in Ph⁺ positive CML.

An extra chromosome 8 appears to be associated with premalignant or malignant hematological diseases engaging myelo- or erythropoietic cells (7 17 18 21). From the present work it appears that the extra C group chromosome sometimes found in the blastic stage of CML may frequently be a number 8 chromosome.

PATIENTS

Seventeen patients with CML were investigated cytogenetically. Eight of the patients were studied before treatment and nine during busulfan therapy. Most of the patients who were on busulfan treatment were analysed at a stage of insufficient treatment and thus at a relatively high WBC. The relevant hematological data at the time of investigation are seen in Table I.

Fourteen patients were at chronic stage of the disease, one of whom (no. 14) was also investigated in the blastic phase. All these 14 patients were Ph⁺ positive. Two further Ph⁺ positive patients (nos. 13 and 16) were investigated in the blastic phase of the disease. One Ph⁺ negative patient (no. 17) was investigated shortly after initiation of treatment, but was apparently already in an early blastic phase (Tables I and II).

METHODS

Bone marrow and blood specimens were investigated in all patients. Chromosome preparations from bone marrow specimens were made directly on fresh aspirates as described previously (24) and from conventional peripheral blood cell cultures after 72 hours incubation.

The Q-banding technique was performed as described previously (6). Metaphases were selected and photo-

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Table I Hematological data at the time of chromosome analysis

Pat. no	Age (y)	Sex	Previous treatment	Hb (g/100 ml)	Platelets $\times 10^{-9}/\text{mm}^3$	WBC $\times 10^{-9}/\text{mm}^3$	Immature cells (%)	Blast cells (%)
1	34	♂	None	11.0	509	164.0	27	5
2	29	♂	None	12.0	120	167.0	37	3
3	40	♂	None	9.2	728	148.0	37	4
4	38	♀	None	7.8	690	86.8	37	8
5	34	♀	None	14.4	422	70.6	28	1
6	44	♀	None	13.9	1 064	20.9	1	0
7	29	♀	None	13.8	1 730	24.5	5	0
8	63	♂	None	12.8	1 200	26.4	3	0
9	19	♀	Busulfan	13.5	388	31.1	14	0
10	27	♀	Busulfan	13.0	180	11.5	3	0
11	23	♂	Busulfan	9.3	532	232.0	36	2
12	31	♂	Busulfan	14.0	335	29.0	17	0
13	41	♀	Busulfan	15.0	391	27.4	16	0
14	32	♂	Busulfan	14.6	173	27.1	22	3
			Busulfan	6.5	13	7.0	66	62
15	25	♀	Busulfan	11.2	446	47.0	22	12
			Benzofen	7.2	26	12.5	76	74
16	21	♂	Busulfan	9.9	107	4.5	68	16
17	71	♂	Busulfan	10.5	539	48.2	40	24



Fig. 1 Chromosomes 9 and 22 in three patients with Ph positive CML showing a typical translocation $t(9q+22q-)$. The normal chromosome pattern in a cell from a healthy person (1) included for comparison.

graphed in a fluorescence microscope and the negative were analysed using a TV set for contrast enhancement (4-5). Only the metaphases which were well spread and had a distinct Q-banding were evaluated. In general the metaphases thus selected amounted to 30-50% of the photographed ones. The number of metaphases evaluated in the bone marrow as well as in the peripheral blood are shown in Table II.

RESULT

The Ph chromosome was found in 16 patients (9 of them have previously been reported in a preliminary communication (11)) (Table II). Chromosome 9 had additional chromosomal material on its long arm in 15 of these cases. The amount of additional material appeared to be approximately the same as that lacking on chromosome 22 (Fig. 1).

Chromosome 9 was normal in one patient despite the presence of the Ph chromosome. As described previously this patient had additional chromosomal material on chromosome 19 (12) suggesting a translocation between this chromosome and chromosome 22. The course of the disease appeared not to be different from that in other Ph positive patients and the survival was about three years (Table II).

One patient was Ph negative. Chromosome 9 was normal (Fig. 2). All other chromosomes also appeared to be normal in this patient.

Three patients (nos 14, 15, 16) with Ph positive CML were investigated in the blastic phase of the

Table II. Chromosome abnormalities stage of disease and survival

C=chronic, B=blastic

Pat. no.	Date of investigation	Stage of disease	No. of metaphases analysed		Chromosomal abnormality	Survival from day of diagnosis
			Bone marrow	Peripheral blood		
1	June 12 1972	C	10	6	9q+ 22q-	595*
2	April 24 1972	C	2	10	9q+ 22q-	746
3	April 17 1972	C	-	5	9q+ 22q-	752*
4	Oct. 1 1973	C	-	23	9q+ 22q-	220*
5	Sept. 25 1973	C	-	7	9q+ 22q-	225*
6	June 19 1973	C	14	-	9q+ 22q-	302*
7	April 24 1974	C	14	-	9q+ 22q-	10*
8	July 22 1974	C	12	-	9q+ 22q-	25
9	Dec. 12 1972	C	15	-	9q+ 22q-	1 494
10	Nov. 17 1971	C	7	-	9q+ 22q-	1 556
11	Jan. 2 1973	C	14	-	9q+ 22q-	531
12	May 8 1972	C	8	-	9q+ 22q-	3 460*
13	Sept. 27 1974	C	13	17	19q+ 22q-	1 090
14	Dec. 20 1972	C	11	5	9q+ 22q-	243
15	Jan. 28 1973	B	3	12	9q+; 22q- 7p- 8+ Ph +	635
	Nov. 6 1973	B	20	2	9q+ 22q- 8+ 11+ 12+ 16+ 17+ 19+ Ph +	
16	Nov. 29 1973	B	-	10	9q+ 22q- 7+ 8+ 11+ 17+ 19+ Ph +	575
	Aug. 24 1971	B	14	-	9q+ 22q-	
17	May 14 1973	B	5	2	Normal	38

The patient is still alive.

disease. One of these patients (no. 14) was investigated both in the chronic and in the blastic phase.

The first investigation in patient 14 was made at a stage when a few blast cells were present in the peripheral blood but with no other hematological signs of a blastic phase. At this time the patient had the common pattern of a $t(9q+ 22q-)$ abnormality in all metaphases investigated but in two of ten he had an additional Ph chromosome. One month later the patient entered a blastic phase with 62% blast cells in the peripheral blood. At this time he had in addition to the previous abnormality ($t(9q+ 22q-)$) an extra chromosome 8 and one chromosome with a shorter than normal short arm ($7p-$) in ten of twelve investigated metaphases in the peripheral blood, and in two of three investigated ones in the bone marrow. All metaphases had now two apparently identical Ph chromosomes (Fig. 3).

Patient 15 was investigated twice in the blastic phase of the disease and had on both occasions several extra chromosomes (Table II). As patient 14 had two apparently identical Ph chromosomes in all metaphases analysed and an extra chromosome 8 in seven of thirteen metaphases in addition to the

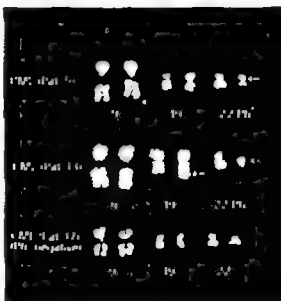


Fig. 2 Normal chromosomes 9, 19 and 22 in patient with Ph negative CML (no. 17). The pattern in Ph positive patient with the $t(9q+ 22q-)$ pattern (no. 5) and in the patient (no. 13) with the $t(19q+ 22q-)$ pattern are included for comparison.



Fig. 3 The karyotype of patient 14 with CML in the blastic phase showing the $t(9q+22q-)$ pattern, an extra chromosome 8, a shorter than normal short arm on chromosome 7 ($7p-$) and two X chromosomes.

$t(9q+22q-)$ abnormality. On the first occasion she had in addition an extra chromosome 19 in all of the eleven evaluable metaphases and also extra 11, 12, 16 and 17 chromosomes in some of the cells. A similar aneuploid pattern was seen about three weeks later (Fig. 4).

The third of the patients in the blastic phase (no. 16) had the $t(9q+22q-)$ pattern and a normal number of chromosomes in the few metaphases investigated the number of which was however not sufficient to make sure that extra chromosomes were not present in some cells.

One patient with CML in early blastic phase (no. 17) was Ph negative. Chromosome 9 as well as all other chromosomes were normal and the number of chromosomes was also normal in all metaphases investigated. The hematological picture was typical of CML. The percentage of mature granulocytes in the peripheral blood was 60 and of myelocytes+metamyelocytes 16. Furthermore the patient had thrombocytosis ($539\,000/\text{mm}^3$) and low alkaline phosphatase score as well as a decreased amount of

PAS-reactive material in neutrophils indicative of CML (10). The number of colony-forming cells in the peripheral blood was characteristically increased in contrast to what is generally found in acute myeloblastic leukemia (14). The course of the disease was rapid and the patient succumbed in a sudden pulmonary oedema probably unrelated to the disease. At that time he had been treated with vincristine and prednisolone and had a hematological picture of an early blastic stage of CML (Hb $10\text{ g}/100\text{ ml}$, WBC $117\,000/\text{mm}^3$, differential count: bands 23.5%, segmented neutrophils 13%, eosinophils 0.5%, lymphocytes 10.5%, monocytes 18.5%, myeloblasts 6.5%, promyelocytes 0.5%, myelocytes 20%, metamyelocytes 7%).

DISCUSSION

It has recently been shown using the Q- and G-banding techniques that chromosome number 9 has additional chromosome material on its long arm in CML (20). This finding has repeatedly been con-



Fig 4 Patient 15 with Ph positive CML, $t(9q+22q-)$, in the blastic phase showing extra chromosomes 7, 11, 19 and two Ph chromosomes.

firmed (1, 2, 11). Although it has not yet been quantitatively assessed it appears that the amount of additional material on chromosome 9 is in most patients about the same as that lacking on chromosome 22. It has therefore been suggested that there is a translocation between chromosomes 9 and 22 in CML (11, 12, 20). However four patients, in whom chromosome 9 was normal despite the presence of the Ph chromosome, have recently been described (9, 12, 14, 19). In one of these patients additional chromosome material was found on chromosome 2 (16) and in the others on the homologous chromosome 22 (9) and on chromosome 18 (12) respectively. In the fourth of these patients it was not possible to find additional chromosome material on any other chromosome using the Giemsa technique (19). It was therefore suggested that this was a true deletion of chromosome 22, but it can not totally be excluded that small amounts of chromosome material had been translocated to a position where it is difficult to detect.

In the present study we report on one further CML patient with a normal chromosome 9. All other chromosomes in this patient were normal and there appeared to be no chromosomes with additional chromosome material. In contrast to the previously

described patients this patient was Ph negative. This observation gives further support to the view that there generally is a true translocation in Ph positive patients although it may involve not only chromosomes 22 and 9 but also chromosome 22 and other chromosomes. Additional chromosome material on chromosome 9 in Ph negative patients would have indicated an aberration independent of the Ph chromosome. This possibility can now be rejected and furthermore it appears that the presence of additional chromosome material on chromosome 9 or other autosomes is of less importance for the development of clinical CML, than the presence of the Ph. In the course of our studies Rowley also found a normal karyotype in Ph negative CML (22) a further support to this view. Non-detectable minute amounts of chromosome material might have been translocated in Ph negative patients, but it is also possible that the normal karyotype reflects on the possibility that Ph negative CML is a disease different from Ph positive.

Chromosome aberrations in addition to the $t(9q+22q-)$ pattern appear to be an early sign of blastic transformation of CML. The most commonly observed aberration is the occurrence of extra chromosomes (22) and duplication of the Ph

chromosome (21). Extra C group chromosomes have been reported in several instances (13-15, 23) and it is of particular interest that in the two patients with aneuploidy the extra chromosome was found to be number 8. Such a finding has previously been made in patients with siderochrestic anemia and acute myeloblastic leukemia (7, 8, 17, 18). In the course of our studies it was suggested that the extra C chromosomes in five CML patients in the blastic phase were identical with chromosome 8 (21). It thus appears that an extra chromosome 8 in hematopoietic cells is in some way associated with hematological malignancies, particularly with those which have a potential for being transformed to a myeloblastic type of leukemia. In CML this chromosome appears late in the course of the disease and indicates the terminal stage.

So far there are no data in the literature as to the frequency of the appearance of the other extra chromosomes noted in this investigation. The current use of the Q-banding and Giemsa-banding techniques may eventually show that a series of specific chromosomal abnormalities may develop during the course of blastic transformation of CML.

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IMMUNOGLOBULIN ANALYSIS IN FAMILIES OF MYELOMA PATIENTS

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Abstract The electrophoretic plasma protein patterns (in search for M-components) and the immunoglobulin (IgG, IgA and IgM) levels have been studied in 24 members of two families with known familial occurrence of myeloma and in 124 relatives of ten patients with myeloma but without known familial occurrence of the disease. Immunoglobulin levels were also determined in control material composed of 34 blood donors of both sexes. In one of the families with multiple occurrence of myeloma a mother and her son had the disease. In the other family two sons had myeloma and one son had an asymptomatic M-component. No further M-component was found among the 24 investigated relatives in these two families. In two of the other ten myeloma families an M-component was disclosed in an asymptomatic relative. The number of M-components found in the present study cannot be considered unduly high, as the prevalence of M-components in an adult population is about 1/100. The distribution of immunoglobulin levels among relatives differed little from that of controls and very few markedly deviating immunoglobulin levels were found. The study thus did not indicate a defective regulation of the immunoglobulin production within myeloma families. Apparently syndromes differs in this respect from Waldenström's macroglobulinemia, which disease has been found preferentially to develop within families in which the monoclonal (IgM) producing cell system is improperly regulated and therefore presumably predisposed to neoplastic transformation.

hypergammaglobulinemia and monoclonal hypergammaglobulinemia, type IgM. This suggests the presence of an improperly regulated and/or hyperreactive light-producing cell system within these families. Such a cell system might be more susceptible to oncogenic stimuli and the familial clustering of Waldenström's macroglobulinemia would thus be accounted for.

Neoplastic plasma cell dyscrasias can be induced or occur spontaneously in certain strains of mice e.g. BALB/c mice which also respond with a stronger and more prolonged antibody response to a standard antigen challenge (26). These experimental results thus support the supposition that an improperly regulated and/or hyperreactive plasma cell system might predispose to the development of neoplastic plasma cell dyscrasias.

Leukemic cells in chronic lymphocytic leukemia have been shown to carry immunoglobulins on their surface and they might consequently be considered as neoplastic B lymphocytes (1) and closely related to the neoplastic cells in myeloma and Waldenström's macroglobulinemia. Familial occurrence of chronic lymphocytic leukemia has long been recognized (29) and in some families immunoglobulin abnormalities have been demonstrated in asymptomatic relatives (8-27) indicating an inherited defect of B lymphocytes within these families.

The present work is an attempt to elucidate whether familial aggregation of myeloma cases might be accounted for by an improperly regulated and/or hyperreactive plasma cell system within myeloma families. The electrophoretic plasma protein pattern (in search for M-components) and the immunoglobulin levels have been studied in two families with known familial occurrence of myeloma.

The possibility of a genetic predisposition to myeloma is suggested by the multiple occurrence of the disease within families (2, 5, 7, 9-11, 19, 21-25, 28, 32, 34). Waldenström's macroglobulinemia, the other major neoplastic plasma cell dyscrasia (26) also clusters within families (31). Furthermore a wide variety of immunoglobulin abnormalities has been found in asymptomatic relatives of patients with Waldenström's macroglobulinemia (15-31). Most frequent of these were polyclonal

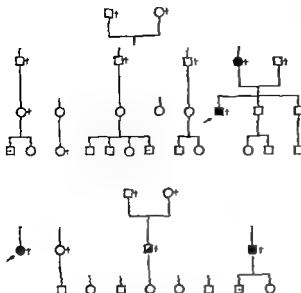


Fig. 1. Pedigrees of families 1.1 (top) and 1.2 (bottom) in material I. □=male, ○=female, ▒=proband, ●=myeloma, ⊠=asymptomatic M-component, □○=non-investigated relative, †=dead.

myeloma and in the relatives of ten patients with myeloma but without known familial occurrence of the disease.

MATERIALS

Material I consists of two families with known familial occurrence of myeloma attending the Department of Internal Medicine, County Hospital of Skövde. In one family (1.1) a mother and her son had myeloma. In the other family (1.2) two sibs had myeloma and a third sib had an asymptomatic M-component. These two families have been previously reported (5). The pedigrees of the families are depicted in Fig. 1.

The proband of family 1.1 had two M-components, IgGK and IgAK. His mother had a single M-component, type IgGK. Neither had Bence Jones proteinuria. The electrophoretic mobility of the M-components was dissimilar. The son had an unequivocal myeloma. Even if the course of the mother's illness was not quite typical of myeloma, this was considered to be the most adequate designation of her plasma cell dyscrasia. She had a rather large M-component (about 30 g/l), albeit stationary for more than 10 years. The proportion of plasma cells in the bone marrow aspirates was 15–20% and clusters of polymorphous and multinuclear plasma cells were also found. The skeleton showed general osteoporosis and collapsed vertebrae but no osteolytic lesions. She had a grave anemia and hepatosplenomegaly. She died shortly afterwards when hospitalized for a traumatic fracture of her left column femoris. No post-mortem examination was performed. Sixteen relatives in family 1.1 were investigated. The relatives of the

father could not be traced. The plasma protein pattern of eight relatives had been examined before the previous report and no abnormalities had been found. Two of these eight relatives later died. No post-mortem examination was performed but there were no indications of myeloma clinically.

The proband of family 1.2 had a myeloma with exclusively light chain myeloma protein, type lambda. One brother had a Bence Jones proteinuria, also type lambda, but of a different electrophoretic mobility. At the time of the previous report he had shown no other clinical signs indicating myeloma. Some years afterwards, five years after the detection of the Bence Jones protein, the patient had multiple osteolytic lesions and about one-fifth of the nucleated cells in the bone marrow aspirate were polymorphous, flaming plasma cells with immunonuclear inclusions. A prominent sharp band of light chains was at the same time seen in the serum electrophoresis. The kidney function rapidly deteriorated and he died in uremia. Another brother had an M-component, type IgGK, that remained stationary and no clinical signs indicating myeloma had appeared when he died, during an episode of hematuria and fever six years after the detection of the M-component. No post-mortem examination was performed. A fourth sib, a sister, had died in cancer corporis uteri some years earlier. The eight children of the sibs were all investigated (Fig. 1). Only a few more distant relatives could be traced and plans for more extensive family study had to be abandoned.

In both the families 1.1 and 1.2 the cause of death of deceased relatives was inquired into. The information gained was usually scarce but in no case were there any indications of myeloma.

Material II consists of the families of 10 myeloma patients attending the Department of Internal Medicine, University Hospital, Lund. At the time of selection of probands there were 24 myeloma patients attending the department. They were all interviewed and informed about the study. If they had a fair number of accessible relatives (6 or more) and if their attitude was positive, they were included in the study as probands.

The probands are characterized in Table 1. Nine had osteolytic lesions, an M-component either in serum or urine and a bone marrow picture indicative of myeloma. One proband had no osteolytic lesions but the bone marrow picture was unequivocal, with 50% plasma cells and a prominent M-component, type IgGL (20 g/l) in serum. Relatives aged 15 years or less were excluded from the study as the immunoglobulin levels during childhood and early adolescence markedly differ from those of adults (33–36) which would have complicated the interpretation of the immunoglobulin levels found during the study. There then remained 130 living relatives, 58 of whom were first degree and 72 second degree relatives. All first degree and 66 second degree relatives have been investigated. Six relatives could not be persuaded to participate. There were no indications that deceased relatives had suffered from myeloma.

Controls. An Australia antigen negative blood donor material composed of 34 adult individuals of both sexes was used as control material.

Table I Clinical and laboratory data of the 10 probands in material II

Proband no.	Sex	Age (y)	M-component		Maximal concentration (approx.) (g/l)		Plasma cells in bone marrow aspirates (%)	Radiological skeletal survey	Relatives (>15 y)			
			Immunological classification ^a						First degree		Second degree	
			Serum	Urine	Serum	Urine			Alive	Studied	Alive	Studied
1	♂	59	IgAL		20		36	Osteoporosis, collapsed vertebrae osteolytic lesions in skull	7	7	8	7
2	♀	55		L		3	61	Lytic lesions in humeri, collapsed vertebrae	8	8	9	9
3	♂	63	IgDL IgGK	L	10 3	1	86	Multiple lytic lesions	6	6 ^b	6	6
4	♀	54	IgGK		50		50	Multiple lytic lesions	4	4 ^b	6	6
5	♀	63		L		14	17	Multiple lytic lesions	2	2	3	3
	♂	64	IgGK	K	5	4	8	Multiple lytic lesions	3	3	3	1
	♂	70		K		6	93	Multiple lytic lesions	5	5	12	12
8	♂	75	IgGK		35		65	Multiple lytic lesions	10	10	12	12 ^c
9	♀	62	IgGL		25		50	Osteoporosis, somewhat irregular	9	9	11	11
10	♂	57		K		3	7	Multiple lytic lesions	4	4	2	0
									38	38	72	66

K=heavy, L=light chains.

^aIn one relative only agarose electrophoresis performed.^bM-component found in one relative.

METHODS

A venous sample (10-20 ml) was drawn either in the laboratory or by local nurse, in which case it was sent by post to the laboratory. Serum was separated within 24-48 hours and stored at -20°C until analysed.

All sera were screened for M-components with agarose electrophoresis (13). When sharp bands, suspected to be M-components, were observed, immunoelectrophoresis according to Schriddeger (30) was performed. Specific antisera to heavy chains (γ and μ) and to light chains (κ and λ) were then used. N quantitative determination of M-components was performed. The concentration of the M-component was, however, estimated by visual inspection of the M-component zone in relation to the albumin and the transferrin zones in the agarose electrophoresis. When an M-component was found by agarose agarose electrophoresis, urine was also submitted to electrophoresis after concentration. Quantitative determination of IgG, IgA and IgM was

performed on all sera except on one, which was spoiled by accident after the performance of agarose electrophoresis. IgG and IgA levels were determined by electroimmunoassay (18). IgM levels were determined by the single radial immunodiffusion method (20). The immunoglobulin levels in the present study are expressed in g/l. One mg immunoglobulin in our reference sera for IgG, IgA and IgM respectively corresponds to 115 (IgG) 39.5 (IgA) and 115 (IgM) potency units of the World Health Organization Serum Immunoglobulin Reference Standard (38).

RESULTS

M-components

No further M-component was found among the 24 investigated relatives in the two families constituting material I (the Skovde families).

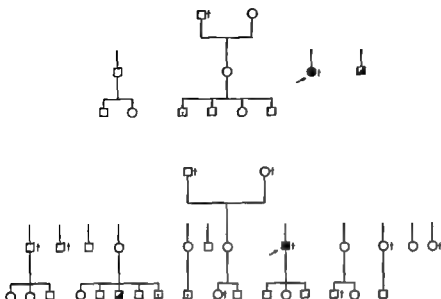


Fig. 2. Pedigrees of families II 4 (top) and II 8 (bottom) in material II. Symbols as in Fig. 1.

In material II an M-component was found in two of the ten investigated families. The pedigrees of these two families are depicted in Fig. 2. The proband of family II 4 had a prominent IgGK M-component. One of her three sons, a 50-year-old brother, had an IgGK M-component, about 10 g/l of slightly different mobility (Fig. 3). The concentration of the M-component has been stationary during a follow up period of two years. Urine electrophoresis has disclosed no Bence Jones protein. Repeated bone marrow aspirates have been essentially normal (maximally 5% plasma cells with no signs of immaturity). Thorough X-ray examination of the skeleton has been normal. He has no anemia and is feeling well.

The proband of family II 8 had also an IgGK M-component (Table I). A 60-year-old nephew had an IgGK M-component of a quite different mobility (the proband had an M-component with cathodal and his nephew an M-component with anodal mobility). The M-component was of low concentration about 5 g/l or less, and has been stationary during a follow-up of two years. He has no Bence Jones proteinuria. There is no anemia and repeated bone marrow aspirates have been essentially normal (maximally 3% plasma cells with no signs of immaturity). Thorough X-ray examination of the skeleton has disclosed no lesions indicating myeloma.

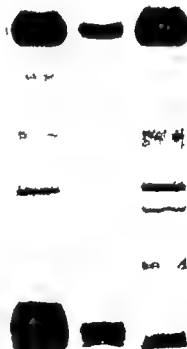


Fig. 3. Agarose gel electrophoresis of serum of the proband of family II 4 (left and middle) and of plasma of her brother (right). The serum sample in the middle was diluted tenfold before application.

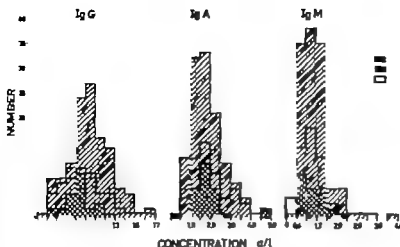


Fig. 4. Distribution of immunoglobulin levels among relatives in materials I (M I) and II (M II) and among controls (C).

Immunoglobulin levels

The distribution of immunoglobulin levels among relatives and controls is depicted in Fig. 4.

The distribution of IgG levels in material I was very similar to that of controls, whereas it possibly differed somewhat in material II from that of controls, the relatives IgG levels tending to be slightly higher. No markedly increased or decreased levels were found, however. The distribution of IgA levels in both materials was similar to that of controls except for a few rather high IgA levels in material II. The distribution of IgM levels in both materials differed little from that of controls. For unknown reasons, however, some remarkably high values were obtained in both materials as well as in controls.

DISCUSSION

The prevalence of M-components in an adult population is about 1/100 (3). All but a minority are asymptomatic (4). In the present study two M-components both asymptomatic were disclosed among 124 relatives of 10 myeloma patients in material II. This number is consequently quite in accordance with the known prevalence figure. In material I, the Skövde families, one asymptomatic M-component was already known at the time of the study but no further M-components were found among 24 investigated relatives, so the number of asymptomatic M-components in these two families cannot be considered unduly high.

Immunoglobulin levels vary with age, sex, race

and environmental influences (6, 12, 14, 33, 36) and such variations should be taken into account when control materials are selected. The blood donors used as controls in the present study were adult individuals of both sexes but differed somewhat from the myeloma material in age distribution (many relatives in the myeloma families being quite elderly), sex distribution (within different age groups) and probably environmental influences (although most controls and most relatives lived in rural areas or in small towns). The controls, however, represented a fair average of a healthy adult population which might be considered sufficient for the purpose of the present investigation, i.e. to elucidate whether the immunoglobulin levels in relatives of myeloma patients indicated an improperly regulated and/or hyperreactive plasma cell system more liable to neoplastic transformation than the plasma cell system of other individuals. If this was the case, one could reasonably expect the immunoglobulin levels in the myeloma families to show a decisive deviation from the average. The distribution of immunoglobulin levels in relatives and controls in the present study was, however, rather similar. Very few relatives had markedly deviating immunoglobulin levels.

Two other systematic surveys of myeloma families have been published, both of which also included probands with M-components from other causes. In the survey of Williams et al. (37) two M-components both asymptomatic were disclosed among 205 relatives of 17 myeloma patients. This number of M-components was thus quite in ac-

cordance with the known prevalence figure of 1%. In the survey of Spengler et al. (32) the prevalence of M-components was about the same with three M-components disclosed among relatives of 14 myeloma patients. One of these M-components was associated with a myeloma, the others were asymptomatic. In both surveys the information on the individual immunoglobulin levels of relatives is scarce. Spengler et al. refrained from carrying out quantitative determinations of individual immunoglobulins as most of the serum electrophoretic and immunoelectrophoretic patterns were normal or approximately normal. Williams et al. performed quantitative estimation of IgG, IgA and IgM on all sera. They report on one myeloma family in which three relatives had high polyclonal IgG levels. They did not comment on the immunoglobulin levels of the other relatives except for the statement that they had found a close quantitative relationship in IgG, IgA and IgM within many sibling groups of the kindreds studied.

The available information from family surveys of myeloma probands including ours thus does not indicate any unduly high frequency of M-components or immunoglobulin abnormalities in myeloma families. Accordingly there is no support for the suggestion of the existence of an improperly regulated and/or hyperactive plasma cell system in myeloma families predisposing to the disease. The possibility can, however not be excluded that a familial predisposition to myeloma exists, which is in fact suggested by a few quite impressive reports on familial myeloma. Thus in a Dutch family two sibs had myeloma and one sib had an asymptomatic M-component. One of the myeloma sibs had one child with myeloma and two children with asymptomatic M-components (24). The immunoglobulin levels in the remaining relatives were essentially normal. In another report two sibs had, and the father might have had myeloma (25). In a third report three sibs had myeloma (2). Most other reported familial myeloma cases are, however the incidental discovery of two family members afflicted with the disease such as our Skövde cases (7-9-11, 13, 21-23, 28-34). Such an observation might be accounted for by chance, as myeloma is not an altogether rare disease, the incidence being about 3/100,000 a year (35). In fact, most myeloma cases are non-familial. Thus among 140 myeloma cases seen by Walden-

ström (35) there were no siblings and of about 550 myeloma cases seen at the Mayo Clinic (10) only two were siblings. The two family surveys referred to earlier indicated only one additional myeloma case among 31 investigated families (32, 37) this being a second degree relative. Nor did our survey disclose any further myeloma cases.

With the exception of a few impressive familial clusterings of myeloma there is thus not much indication of a familial predisposition to the disease. Familial clustering of myeloma, if not fortuitous, might be due as much to common environmental influences as genetically determined. The occurrence of myeloma within four pairs of spouses (16) and a reported geographic clustering of the disease (17) suggest that this could be the case.

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ERYTHROBLASTIC ISLANDS AND INEFFECTIVE ERYTHROPOIESIS IN VITAMIN B₁₂ DEFICIENCY

U Sjögren and L. Brandt

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Abstract. Bone marrow smears from 4 patients with anaemia due to vitamin B₁₂ deficiency have been compared with smears from healthy controls. A three fold increase of erythroblastic islands and predominance of basophilic erythroblasts were found in the patient group. There was a firm, positive correlation between the proportions of basophilic erythroblasts within the erythropoietic pool and the numbers of erythroblastic islands. It is suggested that the large numbers of erythroblastic islands in the patient group are a morphological sign of the ineffective erythropoiesis known to characterize megaloblastic anaemia. The results are compatible with an intramedullary death of especially polychromatic erythroblasts and phagocytosis of these cells by reticulum cells.

Using different techniques, several investigators have presented data that convincingly point to an intramedullary destruction of erythroid precursor cells as an important feature of the ineffective erythropoiesis in megaloblastic anaemia (5, 8, 10, 11). From an electron microscopy study it has been concluded that phagocytic reticuloendothelial cells of the bone marrow may devour erythroblasts in patients with pernicious anaemia (6).

The present investigation was undertaken to find out whether there are any consistent morphological signs in the bone marrow of patients with megaloblastic anaemia pointing to increased erythroblast phagocytosis. Special interest was thus devoted to the presence in the bone marrow of reticulum cells containing cell debris and in contact with erythroblasts "erythroblastic islands" since previous results have suggested that an abundance of such formations may indicate an active phagocytosis of red cell precursors by the reticulum cells which is compatible with an ineffective erythropoiesis (4, 12).

MATERIAL AND METHODS

Patients. Twenty-four patients, 13 women and 11 men, aged 46-87 years (mean 68) with anaemia due to vitamin B₁₂ deficiency were examined. Hb 10-10.5 g/100 ml (mean 7.2), serum B₁₂ 5-90 pg/ml (mean 36), serum iron 11-90 µg/100 ml (mean 19), TIBC 207-320 µg/100 ml (mean 269), MCV 111-140 µ³ (mean 123). Of the erythroid precursors 73-99% (mean 87) were megaloblasts.

Controls. Eleven apparently healthy persons, 6 women and 5 men, aged 41-82 years (mean 64) served as controls. Hb 12.0-16.5 g/100 ml (mean 14.2), all had normal WBC and normal differential counts. ESR was normal in all and no megaloblasts or other abnormalities were found in their bone marrow.

Examination of bone marrow smears. Bone marrow was obtained through conventional sternal puncture and smears were stained with May-Grünwald-Giemsa. 1 000-3 000 (mean 1 160) erythroblasts were classified according to the stage of maturation. Proerythroblasts and basophilic erythroblasts were then pooled into one group designated basophilic erythroblasts. A mitotic index of the erythroblasts was determined and the number of erythroblastic islands per 1 000 erythroblasts was recorded.

RESULTS

The mitotic indices of the erythroblasts were largely the same in the patients and the controls on an average 36 and 31/1 000 respectively (Fig. 1). The composition of the erythroid precursor pool with regard to different stages of maturation differed between patients and controls. Thus the proportion of basophilic erythroblasts was on an average 55% in the patients and 31% in the controls ($p < 0.001$) (Fig. 2).

The average number of erythroblastic islands was 18.2/1 000 erythroblasts in the patient group and only 5.8 in the healthy control (Figs. 3 and 4). The difference is significant ($p < 0.001$).

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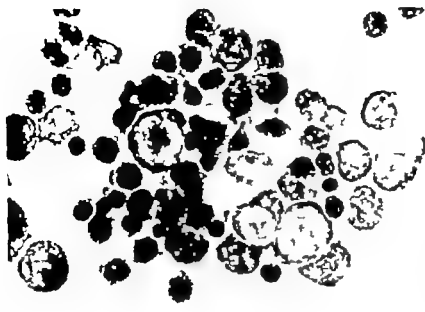


Fig 4 Reticulum cell surrounded by megakaryocytes in a patient with vitamin B₁₂ deficiency

effective erythropoiesis. It has been claimed that the presence of erythroblastic islands in the bone marrow indicates a nursing process in which erythroblasts obtain ferritin from the reticulum cells (3), but it has also been suggested that such formations may indicate an intramedullary phagocytosis and destruction of erythroid cells by the reticulum cells and thus be an expression of ineffective erythropoiesis (4, 12). With regard to megaloblastic anaemia the latter interpretation seems to be the most likely since, as mentioned, intramedullary death and phagocytosis of erythroblasts are typical of this condition.

A firm correlation between the proportions of basophilic erythroblasts within the erythropoietic pool and the frequency of erythroblastic islands in the bone marrow was found in the present investigation. In the patients with the largest proportions of basophilic erythroblasts the erythroblastic islands were also most abundant. As mentioned, polychromatic erythroblasts have a high death rate in megaloblastic anaemia, causing a preponderance of basophilic erythroblasts. It is highly probable that the dead erythroblasts are eliminated through phagocytosis by reticuloendothelial cells. Therefore the presence of large numbers of reticulum cells containing cell debris and in close contact with erythroblasts may be the morphological sign of a high death rate among erythroid precursor cells.

Apart from the megaloblastic changes a very similar pattern showing increased proportions of basophilic erythroblasts combined with increased numbers of erythroblastic islands has previously been found in patients with anaemia associated with inflammatory lesions or malignant disease (4, 17). It is therefore probable that intramedullary death and phagocytosis of erythroblasts may contribute to anaemia also in such patients and that

% BASOPHILIC
ERYTHROBLASTS

60-

80-

40-

20-

6

12

18

24

30

ERYTHROBLASTIC
ISLANDS PER 1000
ERYTHROBLASTS

Fig 5 Relation between the numbers of erythroblastic islands/1000 erythroblasts and the proportions of basophilic erythroblasts within the erythropoietic pool.

high numbers of erythroblastic islands in anaemic patients are a sign of ineffective erythropoiesis irrespective of the aetiology of the condition.

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COAGULATION STUDIES IN HEMOLYTIC UREMIC SYNDROME AND THROMBOTIC THROMBOCYTOPENIC PURPURA

Mats Ekberg, Inga Marie Nilsson and Torsten Denneberg

From the Coagulation Laboratory and Medical Department I, University of Lund General Hospital, Malmö, Sweden

Abstract. Seven patients with hemolytic uremic syndrome (HUS) and 4 with thrombotic thrombocytopenic purpura (TTP) have been studied with almost daily determination of urinary fibrin/fibrinogen degradation products (FDP) and the coagulation and fibrinolytic parameters in blood during the course of the disease. These studies have shown that intravascular coagulation might occur not only in TTP but even in an early phase of HUS. In all these cases there was a good correlation between the clinical course and the increase in platelet count and disappearance of FDP in the urine. The best way of handling the patients with HUS was an early institution of hemodialysis in combination with low dosage heparin. In the patients with TTP the best results were obtained with hemodialysis and adequate continuous heparin treatment.

Disseminated intravascular coagulation or generalized deformation has been reported to occur in hemolytic uremic syndrome (HUS) and in thrombotic thrombocytopenic purpura (TTP) (1, 10, 4, 31). But most coagulation studies of these syndromes have not revealed any signs of consumptive coagulopathy or increased fibrinolysis (9, 18, 23, 28, 34). The efficacy of treatment with heparin has therefore been questioned (16, 23, 36). Treatment of the acute renal failure with dialysis seems to be of utmost importance in these patients (17, 36).

In 7 patients with HUS and 4 with TTP coagulation and fibrinolysis were studied in detail to find out whether these syndromes include intravascular coagulation.

METHODS

The following determinations were made of the platelets, coagulation factors and components of the fibrinolytic system: platelet count, bleeding time (Duke's), recalcification time of plasma, one-stage prothrombin time, prothrombin + factor VII, factor X (Owren's P&P

test), factor V, factor VIII (biological activity and immunoochemical determination), fibrinogen, fibrinolytic activity of plasma and resuspended euglobulin precipitates on fibrin plates, plasminogen (immunoochemical method), fibrin/fibrinogen degradation products (FDP) in serum and urine, and ethanol gelation test. (In other-wise stated, the methods described earlier are used (8, 14, 30)).

Platelet counts were made by the method of Björkman (2).

Factor VIII was immunochemically determined according to Holmberg and Nilsson (15).

Ethanol gelation test was performed according to Godal et al. (1).

FDP in serum and urine were determined by the immunochemical method of Nidén (29) with specific antisera against the D-product using the rocket method of Laurell (22). The serum or urine samples (dil 1/1) were applied to agarose plates containing the anti-FDP serum. On high voltage electrophoresis the antigen is forced into the gel. Precipitation peaks form and their heights are proportional to the amount of antigen in the sample. High molecular weight degradation products were used as standard (13).

Platelet survival studies were performed in the way described by Ljungqvist and Bergentz (26). The ^{51}Cr activity in platelet-rich plasma was measured repeatedly for 48 hours after infusion of ^{51}Cr -labeled normal platelets.

Fibrinohar activity of the vessel wall was studied by the immunochemical method described by Pasdoff (31).

Dialysis treatments. The children were given treatments with disposable plate kidneys of total surface of 0.3 or 0.16 m². The blood tubing and dialyser were primed with blood (about 120 ml), to which 1000 U heparin/1000 ml had been added. For the adult, hollow fiber kidney was used as dialyser. A dialysate flow of 1 l/min, blood flow of 100-200 ml/min and ultrafiltration of 60-700 mmHg were used.

Coagulation. The astatic-tellon arteriovenous cannula system described by Quenon et al. (33) was placed in the radial artery and in the cephalic vein. In one patient the brachial artery was cannulated.

Parenteral nutrition. During the acute stage of the disease the patients were nourished with essential amino

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9	10	11	Normal range
4	9	9	
31	49	25	
10.0	13.0	0.9	0.9-1.2
380	250	250	200-400 000
4 000	42 000	64 000	
18	0	16	
			40-175
213	208	370	60-160
		-	60-175
90	128	28	80-120
63	100	32	80-120
0.62	0.32	0.20	0.20-0.40
77	50	750	0-5
	70	50	0
	Pos.	-	Neg.
96	120	15	70-130
	Steroid	HD	
HUS Malign. hyper.	HUS SLE	Postpart. ren. fail. Defibr	

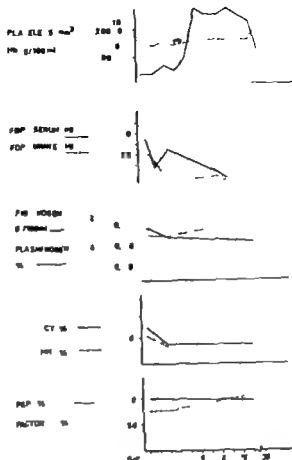


Fig 1 Case 1

ment with hemodialysis 4 times. After 3 days the production of urine started and renal function successively became normal.

Case 3 (Fig 3)

A 1-year-old girl. Four to five days before admission to hospital she vomited and had diarrhea. Disseminated purpura. No urinary production. Hb 7.8 g/100 ml, platelets 26 000/ μ l, reticulocytes 74 000/ μ l, serum creatinine 3.1 mg/100 ml. During the first few days the patient had repeated attacks of convulsions. Treatment with hemodialysis was started. To prevent clotting in the shunt, heparin was given (250 IU \times 4) from the 3rd to the 9th day as well as about 1 500 IU during treatment with hemodialysis 6 times, about 4 hours each time. The patient's condition was extremely poor and she had repeated attacks of convulsions, but after 10 days the urinary production increased, dialysis was stopped, and the patient recovered.

Case 4 (Fig 4)

A girl, aged 2 $\frac{1}{4}$ years, was admitted to hospital after 6 days diarrhea and vomiting and no production of urine during the last 3 days. Physical examination pale apathetic girl in poor condition with repeated

seizures. Hb 5.9 g/100 ml, platelets 42 000/ μ l and serum creatinine 10 mg/100 ml. Th blood film revealed Burr cells. During the first days the patient required peritoneal dialysis, and on the 3rd day hemodialysis was started, which was given also on the 4th and 6th day. Because of clotting in the shunt low dosage heparin (1 000 IU/day) was given besides the heparin administered during the dialysis (2 000 IU during the 5-hour dialysis). On the 6th day of treatment the urinary production increased and dialysis was no longer necessary. The patient recovered.

Case 5

A girl, aged 3 years, had always felt well until 1 week before admission, when she developed fever with vomiting, abdominal pain and diarrhea. On admission dry scannable petechiae and ecchymoses. Hb 5 g/100 ml, platelets 6 000/ μ l, reticulocytes 6,000/ μ l. Increased number of megakaryocytes in the bone marrow. Haptoglobin 6 mg/100 ml. Proteinuria and hematuria, but serum urea and creatinine were within normal limits. Studies of the erythrocyte survival with 51 Cr-labeling revealed pronounced hemolysis. The patient was given large doses of steroids with no effect. Splenectomy was performed and azathioprine was administered. The pa-

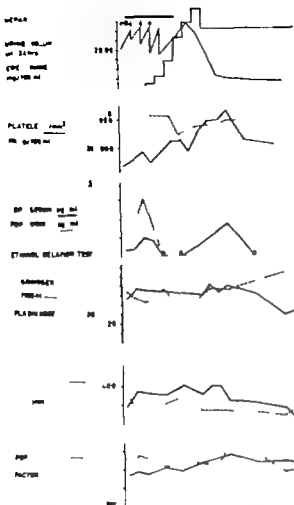


Fig 2 Case

treat was given large amounts of fresh blood. Neither the Hb nor the platelet count increased. The patient's condition had until then been fairly good. But renal function was now impaired and the BP rose. Paresis of the left arm and leg appeared and she had repeated seizures and died. Autopsy showed severe changes in the vessels of precapillary caliber in the form of endothelial hyperplasia, florid necrosis and microthrombi in most of the organs.

Group II (cases 6-11) consisted of 4 women and 2 men. Two women and two men with malignant hypertension and involvement of multiple organs before admission. Their renal function deteriorated when the disease was complicated by development of TTP. One woman who had had SLE for many years and one woman with postpartum renal failure developed HUS. Two of the patients with malignant hypertension (nos. 6 and 7) and the woman with postpartum renal failure were given hemodialysis treatment during which they were heparinized. In these patients heparin was administered also between the dialyses intermittently or as a continuous infusion.

Case 6 (Fig 5)

A woman, aged 48, had always felt well until the end of 1971, when progressive occipital headache, dizziness and blurred vision occurred. On admission petechiae on the legs and peripheral edema were seen. Papilledema, hemorrhages and exudates were demonstrated in her fundi. BP 50/110 mmHg, Hb 9.7 g/100 ml, platelets 20 000/ μ l, haptoglobin 7 mg/100 ml. The blood film revealed anisocytosis, polikocytosis and fragmented red cells. Serum bilirubin 1.6 mg/100 ml, serum creatinine 1.1 mg/100 ml. Platelet survival studies with ^{51}Cr -labeled normal platelets showed a half-life of about 30 min. Fluctuating neurological signs and paresis of the left arm and leg developed. Hemodialysis was started because of oliguria with grave electrolyte deficits and pulmonary edema. Continuous heparin infusion with 20 000 IU/day was started and the platelet count rose to 110 000/ μ l but decreased to 15 000/ μ l on the following days when heparin was withdrawn. The platelet count returned to normal and the coagulation factors improved during heparin infusion, which was continued for 5 weeks. After 7 treatments with hemodialysis the urine output increased but the serum creatinine persisted at a level of 5-6 mg/100 ml. The hypertension was caused by a stenosis of the right renal artery. Renal biopsy of the left kidney showed severe arteriolar sclerosis. After nephrectomy of that kidney

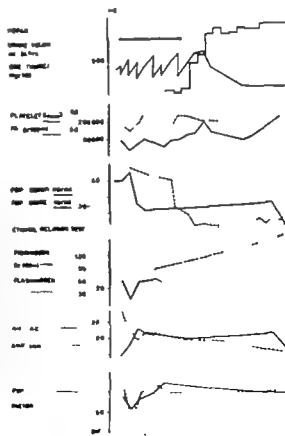


Fig 3 Case 3

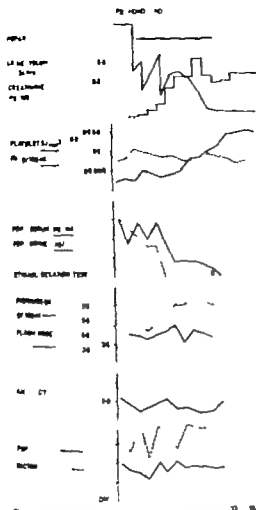


Fig 4 Case 4

and reconstruction of the renal artery stenosis, the renal function and BP became normal.

Case 7 (Fig 6)

A woman, aged 51, was admitted to hospital after 3 weeks of abdominal pain and vomiting. Laparotomy revealed gangrene of the small bowel caused by multiple thrombi in the mesenteric vessels. Postoperatively thrombus developed in v.v. portae and multiple thrombi in the arms and legs. Hb 5 g/100 ml, platelets 4000/ μ l, haemoglobin 0 BP 250/140 mmHg. Examination of the ocular fundi revealed hemorrhages, exudates and papill edema. Serum creatinine 4.6 mg/100 ml. Hemodialysis was started because of oliguria and decreasing renal function. The patient was treated 13 times with hemodialysis, about 6 hours on each occasion, during which 15-20 000 IU heparin was given. Heparin was administered subcutaneously 12 500 IU twice daily. The platelet count rose and the renal function and various coagulation factors became normal during the treatment. After some months, however, the patient died from abdominal and pulmonary abscesses.

Case 8

A 33-year-old man was admitted to hospital because of severe occipital headache during the previous few weeks. On admission BP was 190/130 mmHg, the heart size as increased and ocular fundi showed exudates and hemorrhages. Hb 10.0 g/100 ml, platelets 4000/ μ l, reticulocytes 1.4000/ μ l, haemoglobin 0. Serum creatinine was 3.0 mg/100 ml and rapidly increasing because of oliguria. During the following week multiple thrombi developed in the legs and cerebrum and the patient died. Autopsy revealed severe arteriosclerosis, changes in the vessels with multiple thrombi.

Case 9

A 51-year-old man with hypertension known for 2 years with cardiomegaly and changes in the fundi corresponding to KW III. During this time serum creatinine had been stable at about mg/100 ml. He was admitted to hospital because of deterioration. Hb 9.7 g/100 ml, platelets 4000/ μ l, haemoglobin 0 BP 200/140 mmHg and serum creatinine 10 mg/100 ml. The patient became oliguric and died from pulmonary edema.

Case 10

A woman aged 49 with known SLE with an onset of multiple organ failure since 1954. Until admission the serum creatinine level had been about mg/100 ml.

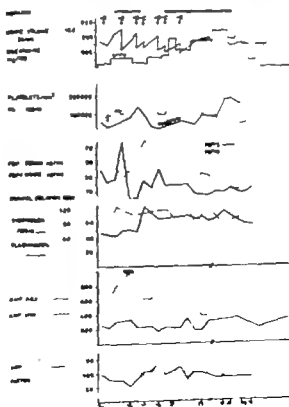


Fig 5 Case 6

admission multiple petechiae and ecchymoses. Hb 9.8 g/100 ml platelets 4 000/ μ l reticulocytes 778 000/ μ l and haptoglobin 0, serum creatinine 13 mg/100 ml. The patient became oliguric and was treated with peritoneal dialysis bathed.

Case 11 (Fig 7)

A 35-year-old pregnant woman who had felt well until 1 month before calculated delivery when she became icteric. Serum bilirubin 8 mg/100 ml the transaminase values were high and the alkaline phosphatases considerably increased. Caesarean section was done because of the impaired liver function. Examination of the various coagulation factors revealed a massive consumption. Hb 9 g/100 ml platelets 64 000/ μ l haptoglobin 16 mg/100 ml and serum creatinine 3.7 mg/100 ml. The patient received 20 000 IU heparin on the first day and 30 000 IU on the second day after operation as a continuous infusion, besides multiple transfusions of fresh blood. Oliguria-azuria ensued. The

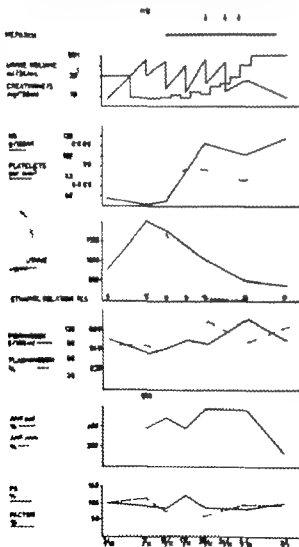


Fig 6 Case 7

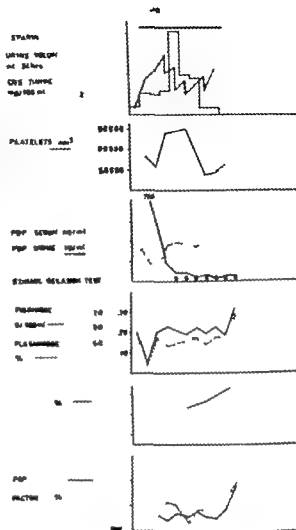


Fig 7 Case 11

heparin supply was continued and treatment by hemodialysis was started during the following day she was treated 4 times. During treatment the fibrinogen level rose and the platelet count and the coagulation factors became normal. However the patient died of acute liver necrosis.

RESULTS

The results of coagulation analyses and some other relevant data on admission are summarized in Table I.

All the 11 patients had low platelet counts, hemolysis and renal damage of varying severity. The initial levels of factor VIII as determined by the biological method were high in all patients except one. Factor VIII determined immunologically in 4 patients was extremely high in all. Ten patients

were examined for FDP which was invariably found in large amounts both in serum and urine. On admission only one patient (no. 11) with post partum renal failure showed multiple defects with low levels of P&P factor V, fibrinogen and plasminogen. Of the remaining 10 patients 8 had normal and 2 high P&P values, factor V was normal in 7 and markedly increased in 3. The fibrinogen levels were either normal or high. The plasminogen level was normal.

None of the patients showed increased fibrinolytic activity in the circulating blood assayed on fibrin plates. The ethanol gelation test performed in the initial stage in five patients, was positive in only one (no. 10).

All patients in group I had symptoms of HUS which were kept under observation by daily determinations of the various coagulation and fibrinolytic components (Figs. 1-4). Patient 1 received no heparin and hemodialysis was not performed. As is seen from Fig. 1 the platelet count increased to normal and the FDP disappeared within one week which coincided with clinical recovery. Patients 2, 3 and 4 were treated almost daily with hemodialysis. Heparin in very small doses was given for a short time to prevent clotting in the shunts. The platelet counts became normal and the FDP in the urine disappeared. Only in case 3 (Fig. 3) could a marked decrease in the various coagulation factors be demonstrated on the second day after admission. On the following day the values again began to increase. In all these cases there was a good correlation between the clinical course and the increase in platelet count and disappearance of FDP in the urine.

Case 5 presented the clinical symptoms of HUS already on admission. Throughout the disease the fibrinogen level was normal, P&P factor V and factor VIII were increased and the platelet count was low. FDP were present in serum but were not determined in urine. She received no heparin or hemodialysis treatment. TTP developed with multiple thrombi in all organs and the patient died.

In group II the patients had complicated latent diseases. In two patients with malignant hypertension and TTP (nos. 6 and 7) platelet survival studies and almost daily coagulation studies were performed (Figs. 5 and 6). Both patients were found to have a rapid consumption of platelets, the half-life being only 1/2-1 hour. Initially both had normal fibrinogen values but high FDP levels

both in serum and urine. Factor VIII was also markedly increased while the other coagulation factors were largely normal. The patients were treated with heparin and hemodialysis. The course in case 6 is shown in Fig. 5. During treatment the platelet count and the fibrinogen increased while the concentration of FDP decreased. A similar course was seen in case 7 (Fig. 6). In both cases renal function became normal.

In cases 8 and 9 with malignant hypertension and in case 10 with SLE we followed only the platelet count. These patients received no hemodialysis or heparin treatment. The platelet count remained low throughout the disease. The level of the various coagulation parameters found on admission did not differ from those in the other patients in this group. All these patients died in uremia. Patient 11 (Fig. 7) initially showed multiple coagulation defects. She was treated with heparin and dialysis and the platelet count, coagulation factors became normal but as the disease progressed and the patient died in liver necrosis and uremia.

DISCUSSION

There is no agreement on the role played by disseminated intravascular coagulation in the pathogenesis and symptomatology of HUS and TTP. Anticoagulation with heparin has been employed with varying success in patients with this syndrome (10-36). In most reported cases no detailed studies of the coagulation and fibrinolytic factors have been performed throughout the disease though such studies are important for evaluating the role played by intravascular coagulation and anticoagulation treatment in these syndromes.

The five patients in our group I fulfilled the criteria of HUS. All of them were followed by daily determination of the various coagulation and fibrinolytic factors. They had normal or raised levels of factors V and VIII, P&P and fibrinogen. FDP in serum and in urine were demonstrated in high concentrations but no other signs of increased fibrinolysis in the circulation. These results are in agreement with a local coagulation with fibrin deposits in the kidneys rather than intravascular coagulation, as was also found by Katz et al. (18) and Lieberman (23). In case 3, however, consumption of the coagulation factors was demonstrated the platelet count in the veins

of fibrinogen, factor V, factor VIII and P&P decreased significantly, but fibrinolysis was not increased. The patient's condition deteriorated with fluctuating neurological symptoms and seizures which disappeared when coagulation factors and platelet count improved during continued treatment (Fig. 3). The occurrence of generalized defibrination can apparently occur in an early phase of these syndromes. This has also been suggested in earlier publications, but detailed sequential studies of the coagulation factors were not performed (1, 10).

TTP has been described in combination with malignant hypertension (5, 7, 19, 25, 35). The severe arteriolar damage occurring in malignant hypertension and even in collagen diseases such as SLE, expressed as hyperplasia of the intima and proliferation of the endothelial cells and fibrinoid necroses, might produce abnormalities in the microcirculation, with the development of disseminated intravascular coagulation and microangiopathic hemolytic anemia as a result (19). Similar vessel changes have been described in pre-eclampsia and postpartum renal failure (19).

Four of our patients in group II had malignant hypertension. At least three of them developed TTP accompanying their hypertension and renal damage. They had a rapid consumption of platelets. The fibrinogen level was normal in spite of the clinically acute disease, indicating an increased consumption. Two were treated with heparin, which was followed by normalization of the platelet count, increase in fibrinogen, disappearance of FDP and improvement of renal function (Figs 5 and 6).

In case 11 the FDP level was very high and fibrin monomers could be demonstrated in the blood. The low coagulation factor levels might be due to a deficient synthesis because of the liver damage, or indicate an activation of the coagulation process. The high FDP level in serum is remarkable. A vessel biopsy examined by the histochemical method of Pandolfi (31) showed no occurrence of fibrinolytic activators in the vessel wall, indicating that the patient had emptied most of the fibrinolytic activators from the vessel walls to resolve the fibrin deposits occurring in most of the organs. Treatment with heparin in this case successively normalized the coagulation factors.

The pathogenesis of HUS and TTP are probably identical and only represent various degrees

of manifestation of the same disease. In HUS there are usually only symptoms of thrombi and fibrin deposits from the kidneys, resulting in various degrees of renal damage. The syndrome can, however, result in involvement of several organs, indicating occurrence of TTP with disseminated thrombi of the microcirculation. In case 5 it was apparent that the HUS might proceed to a generalized involvement of multiple organs, which was verified at autopsy.

In most cases of HUS autopsies show intrarenal thrombosis, while TTP is accompanied by severe arteriolar disease and disseminated thrombi of fibrin in most organs (20).

As a rule no consumption coagulopathy has been demonstrated in the syndromes discussed, except rapid consumption of platelets (3, 4, 9, 41). Detailed coagulation studies in our patients have, however, shown that intravascular coagulation occurs not only in TTP but also in an early phase of HUS, as was also shown by Corrigan et al. (6).

Opinions differ on the effect of heparin in HUS. According to some authors (10, 1, 3) treatment with heparin can reduce the mortality rate among children with this syndrome, while others have found no such effects of heparin (11, 7, 36).

Case 1 had a very mild form of HUS which did not require treatment and healed spontaneously. Case 5 was treated neither with dialysis nor heparin; the disease developed into TTP and the patient died from severe neurological and renal complications. The other three patients in our group I with HUS were given treatment with low dosage heparin in order to prevent clotting in the external shunts. The quantity of heparin given in these cases was so low that it had no prolonging effect on the coagulation times. The possibility of heparin preventing a continued activation of the coagulation process can, however, not be excluded. The low dosage heparin might here act in the same way as in the prevention of complications with postoperative thrombosis by preventing the activation of factor X.

Early institution of hemodialysis is probably of utmost importance in severe cases of HUS. When our patients recovered, their coagulation states became normal. Accordingly, in our experience the best way of handling patients with HUS is an early institution of hemodialysis in combination with low dosage heparin. In this way the uremic

symptoms and the nutritional problems of the patient can be handled in an effective way and a continued activation of the coagulation process can probably be prevented. If the disease proceeds, with the appearance of neurological symptoms or involvement of multiple organs adequate heparinization even between treatments with hemodialysis should be instituted promptly.

ACKNOWLEDGEMENTS

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SUCCESSFUL IMMUNOSUPPRESSIVE TREATMENT OF OLIGURIC EXTRACAPILLARY GLOMERULONEPHRITIS

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Abstract Ten consecutive patients with oliguric extracapillary glomerulonephritis have been treated with prednisone and/or azathioprine. Six patients responded favourably to the treatment, while 4 died of uremia. Of the 6 responders 5 were treated with prednisone and azathioprine and one with prednisone. The 6 patients are still on immunosuppressive treatment and have creatinine clearances of 1-75 ml/min 2-43 months after start of treatment. Thus treatment with prednisone and azathioprine seems to improve the prognosis of oliguric extracapillary glomerulonephritis.

The clinical course of extracapillary glomerulonephritis is that of a rapidly progressive disease which in untreated cases ends fatally within a few months or less (1-4). There have been few reports of immunosuppressive treatment of this primary renal disease—which lacks the systemic involvement found in Goodpasture's syndrome, Wegner's granulomatosis, polyarteritis nodosa and systemic lupus erythematosus—and no substantial evidence of improvement in renal function due to the immunosuppression has been reported (2, 5-7). In the present investigation 10 patients with primary oliguric extracapillary glomerulonephritis were treated with prednisone and/or azathioprine which led to a marked improvement in renal function when the two immunosuppressives were administered simultaneously.

MATERIAL

The material comprised 10 patients, 4 females and 6 males, aged 7-61 years. The clinical picture in all patients was very similar. The initial complaints were arthralgias and gastrointestinal symptoms, and in no case were there such renal symptoms as haematuria, oedema or hypertension.

On admission the duration of symptoms ranged from

4 to 60 days. All patients were oliguric and uraemic. Endogenous creatinine clearance was 0-3 ml/min and BP 125/80-190/120 mmHg. Chemical and laboratory data are given in Table I.

Antistreptolysin titre was increased (≥ 200) in 3 patients (nos. 4, 6 and 10) and normal (< 200)

Pathological changes

Renal tissue was obtained from 9 patients by 4 and from one patient (no. 1) at autopsy. In each two biopsies were performed at an interval of 30 d. (Table II).

In the initial biopsies from the 10 cases "reactive" changes generally cellular were found in 60-100% of the corpuscles examined. Collagen organization was present in 50% of the crescents in cases 4 and 7 only. Recent changes comprising focal necrotic areas, fibrin deposit or infiltration with polymorphonuclear leucocytes were frequently seen in both the crescents and the glomeruli. In the latter there were locally slight basement membrane thickenings. Few or no hyalinized glomeruli were present. Some hypercellularity of the glomeruli was found in 9 of the 11 biopsies.

Slight to moderate tubular atrophy and slight interstitial fibrosis was seen in 3 biopsies. In the interstitial tissue the presence of oedema and some infiltration with polymorphonuclear leucocytes and lymphocytes was more frequent finding. There were no signs of arteritis and no morphological changes consistent with collagen disease.

In patient 7 from whom the first biopsy was obtained just before the combined immunosuppressive treatment was initiated, crescent formation was found in 82% of corpuscles. None of the crescents were hyalinized. Necrotic changes were seen in both the crescents and the glomeruli. In the second biopsy the frequency of crescents was reduced to 48% of the corpuscles and in 4 of 11 crescents there were hyaline changes. At this time no necrotic lesions were observed in the crescents or the glomeruli (Fig. 1).

Treatment

Patients 1-3 were treated with prednisone 4 and 5 with azathioprine and 6-10 with prednisone and azathioprine.

Table 1 Immunosuppressive treatment

Pat no	Sex	Age (y)	Duration of disease before treatment (d.)	Days before response or death	Endogenous creatinine clearance (ml/min)			Proteinuria (g/24h)			Time of follow-up (mo.)
					At start of treatment	1 mo. after treatment	At latest follow-up	At start of treatment	1 mo. after treatment	At latest follow-up	
1	♂	19	30	12 (†)	—	—	—	0.1	—	—	—
2	♂	45	45	15	2	27	52	1.5	3.2	1.1	43
3	♀	56	60	18 (†)	—	—	—	0.2	—	—	—
4	♂	9	45	45 (†)	2	—	—	1.8	—	—	—
5	♂	17	30	52 (†)	—	—	—	2.5	—	—	—
6	♀	7	14	10	—	25	21	1.0	4.0	5.5	4
7	♂	32	30	14	—	70	75	0.4	0.7	2.1	15
8	♂	37	25	24	3	33	30	4.0	2.0	3.0	—
9	♀	49	30	9	—	31	53	0.2	1.5	1.1	8
10	♀	61	4	15	—	30	48	0.3	2.0	1.1	11

thioprine (Table 1). In the adult patients the initial dosage of prednisone was 80 mg/day and in the children 30 mg/day. Azathioprine was given in a dose of 2 mg/kg b.wt./day. At the most recent follow-up

all responders were still on immunosuppressive treatment with azathioprine (2 mg/kg b.wt./day) and/or prednisone (3–30 mg/day).



Fig. 1 Renal biopsies from patient 7. In the initial biopsy (a) two corpora with cellular crescents are seen. Interstitial oedema is present between the tubules (H and E $\times 40$). In the second biopsy one month later



(b) slight interstitial fibrosis is found. In one of the two corpora the arcuate matrix is augmented and the crescent is hyalinized (H and E $\times 140$).

RESULTS

Only one (no. 4) of three patients treated with prednisone responded to the treatment (Table 1). At the most recent follow-up—43 months after start of the treatment—this patient had an endogenous creatinine clearance of 57 ml/min, a proteinuria of 1.1 g/24 h and a normal BP.

Both patients (nos. 4 and 5) treated with azathioprine died without increased diuresis 45 and 52 days after start of the treatment.

All 5 patients (nos. 6–10) treated with prednisone and azathioprine responded to the treatment in the course of 9–24 days. At the most recent examination—2–15 months after the favourable response—endogenous creatinine clearance was 21–75 ml/min, proteinuria 0.3–5.5 g/24 h and BP 120/60–220/120 mmHg.

DISCUSSION

Except in a very few cases (3) it has generally been accepted that oliguric extracapillary glomerulonephritis progresses to terminal uraemia in the course of weeks or months (2, 4, 6).

Only a few reports of immunosuppressive treatment of oliguric extracapillary glomerulonephritis are available. Thus Nakamoto et al. (7) reported glucocorticoid treatment of oliguric glomerulonephritis. At most 5 of their 9 patients suffered from the extracapillary type of glomerulonephritis and only 2 of these 5 patients responded to glucocorticoid treatment with improvement in creatinine clearance. Two patients with "crescent formation" in renal biopsy reported by Kincaid-Smith et al. (5) responded favourably to a combined treatment with glucocorticoid, azathioprine and anti-coagulants. However the percentage of corpuscles showing crescent formation was not indicated and it is therefore difficult to make a comparison with our material.

In the present material crescent formation was found in 60% or more of the corpuscles. Of the 3 patients treated with prednisone alone one responded with improvement in glomerular filtration rate (Table 1). Both patients treated with azathioprine died from uraemia. However all the 5 patients treated with a combination of prednisone and azathioprine achieved a substantial increase in creatinine clearance. There was no systematic difference in the severity of histological findings and clinical parameters including

Table 11 Glomerular lesions
+ = sparse, ++ = moderate, +++ = pronounced
■ = 70% or more

Pat. no.	No. of corpuscles	Crescents					Glomeruli				
		Frequency (%)	Epithelial cell proliferation	Fibrinoid changes	Presence of polymorphs	Fibrin deposits	Necrotic changes	Hypertrophy	Presence of polymorphs	Fibrin deposits	Necrotic changes
1	>100	76	+++	++					+	+	+
2	15	86	+++	++				++	++	++	++
3	10	60	+++	++				++	++	++	++
4	96	100	+++	++	+	+	++	++	+	+	++
5	2	100	+++	++			++	++	+		++
6	20	93	+++	++			++	++	+	+	++
7	12	82	+++	++	+	+	++	++	+		++
7b	23	48	+++	++			++	++	+	+	++
8	26	69	+++	++			+	++	++	+	++
9	20	75	+++	++	++	+	+	++	++	+	++
10	14	79	+++	++			+	++	++	+	++

evidence of antecedent streptococcal infection between the patients who responded to the treatment and those who died from apparently irreversible uraemia.

In patient 7 from whom a second biopsy was obtained after one month of treatment, a reduction occurred in the percentage of corpuscles with crescent formation, as well as in other recent glomerular changes. Thus in this patient the morphological changes paralleled the clinical course.

In spite of the small number of patients investigated, the uniformly favourable response in 5 consecutive patients treated with a combination of azathioprine and prednisone seems to indicate that the course of oliguric extracapillary glomerulonephritis can be improved by immunosuppression.

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THE FUNCTIONAL PATTERN IN PATIENTS WITH RENAL DISEASE

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Abstract: The glomerular filtration rate (GFR), the effective renal plasma flow (ERPF), and the filtration fraction (FF) have been studied in 38 patients with different renal diseases and varying degrees of renal insufficiency. All diagnoses were verified by percutaneous renal biopsy. Eighteen living kidney donors comprised a control material. In 10 patients in terminal uremia no characteristic functional pattern was found. Twelve patients with interstitial nephritis had a significantly increased FF. 9 with the nephrotic syndrome had a significantly decreased FF, whereas 7 with glomerulonephritis without the nephrotic syndrome did not demonstrate FF values different from those found in normals. A good correlation was found between the size of the FF and the concentration of serum albumin. Creatinine clearance overestimated the true GFR in patients with renal disease. The FF was not significantly increased in hypertensive patients.

these physiological methods of study have never had any importance for clinical diagnosis.

It should however be noted that diagnostic classification in these studies was made solely on the basis of clinical criteria and that these studies were carried out before the introduction of percutaneous renal biopsy. Use of this technique has made an essential contribution to precise and clinical diagnosis *in vivo*.

The purpose of the present study was to investigate the functional pattern in patients with different renal diseases and varying degrees of renal insufficiency in whom diagnosis had been verified by percutaneous renal biopsy.

MATERIAL

Fifty-six individuals were studied. Eighteen of these were living kidney donors and served as a control material (21). Of the 38 patients, aged 14-75 years, 21 were women and 17 men. All had renal disease and creatinine clearance varying from 1.58 to 86 ml/min.

Patients were divided into the following groups: Group I, 10 patients in terminal uremia of varying etiology (Table I). Group II, 12 patients with interstitial nephritis—of whom 9 had non-obstructive pyelonephritis without bacteriuria and 3 "analgetic" nephritis. Group III, 9 patients with the nephrotic syndrome. In 5 patients the basic disease was proliferative glomerulonephritis and in 2 membranous glomerulonephritis. In 2 patients minimal lesion or no change was found. Group IV, 7 patients with chronic glomerulonephritis without the nephrotic syndrome, of whom 4 had proliferative glomerulonephritis and 3 membranous glomerulonephritis. There were no patients with obstruction of the excretory renal tract.

Among the 28 patients in groups II-IV 14 had hypertension, of whom 7 in group II with interstitial nephritis, 3 were patients with the nephrotic syndrome and 4 with chronic glomerulonephritis. None of the patients were clinically overhydrated. None received dialysis.

The development of clearance techniques in the thirties (22-23) which made it possible to evaluate glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) separately was followed by attempts to investigate whether various "renal disease entities" were accompanied by characteristic patterns of function. Most of these studies were carried out in patients with glomerulonephritis, pyelonephritis or nephrosclerosis and were undoubtedly motivated by an effort to contribute to diagnostic classification. In patients with post-streptococcal glomerulonephritis a reduced filtration fraction (FF) was demonstrated (4-7). In patients with chronic pyelonephritis and minimal hypertension an increased FF was found (6). Similar observations were obtained in patients with hypertension, particularly malignant (9-10, 13). Rasmussen (17) found that an increased FF was not characteristic of patients with chronic pyelonephritis and hypertension. In general the results of these studies did not demonstrate any specific or characteristic functional patterns and therefore

Table III. Kidney function studies in patients with the nephrotic syndrome

Abbreviations as in Table I

Pat. no.	Sex	Age (yr)	C _{in}	C _{cr}	C _{cr} /C _{in}	C _{125I-PAH}	FF C _{cr} /C _{125I-PAH}	FF C _{cr} /C _{125I-PAH}	Proteinuria (g/24 h)	S-albumin (g/100 ml)	Hct (%)	RF
23	♀	49	11.0	16.3	1.48	63	17.5	5.9	7.5	3.3	29	210/110
4	♂	28	18.9	27.9	1.48	87	1.7	32.1	10.0	2.2	36	170/120
25	♀	47	70.8	65.0	3.12	120	17.3	54.3	70.0	0.8	38	135/100
26	♀	32	28.3	48.4	1.71	178	15.9	27.2	17.5	2.4	35	190/105
77	♀	48	22.1	58.0	2.6	277	8.0	20.9	19.0	0.5	35	140/100
28	♂	55	35.0	55.0	1.57	265	13.2	20.8	15.0	1.0	31	120/90
79	♂	42	37.3	58.5	1.56	332	11.2	17.6	35.0	0.7	48	140/90
80	♂	54	43.0	73.0	1.70	440	9.8	16.6	11.0	0.5	29	120/70
11	♂	43	86.0	125.0	1.45	592	14.5	21.1	19.0	1.9	39	140/90
Average		42	33.6	58.6	1.85	261	14.3	26.3	17.0	1.4		
S.D.			22.1	30.6	0.60	174	4.3	11.6	8.0	0.8		
S.E.M.			7.4	10.2	0.20	58.1	1.4	3.9	2.7	0.7		

chronic glomerulonephritis ($p < 0.01$). Fourteen patients had hypertension. 14 were normotensive. FF among the hypertensives was $26.8\% \pm 9.5$ whereas the value for the normotensives was $7\% \pm 9.6$. The difference is not significant.

DISCUSSION

Determination of GFR and ERPF in all groups showed good agreement between inulin and ¹²⁵I-iothalamate clearances and between PAH and ¹²⁵I-hippuran clearances. Thus renal handling of these materials appears to be identical even under pathological conditions (8, 19, 20).

The reason for the change in relationship between GFR and ERPF could be damage to the glomerular apparatus, changes in colloid osmotic

pressure or lesions of the tubular apparatus (21). The high FF values in patients with interstitial nephritis show that damage to the tubuli dominates (Table II). PAH transport per nephron has been shown to increase with decreasing nephron population in non-uremic animals (3). If tubulus cells from rats and rabbits are treated with azotemic serum, the ability of these cells to accumulate PAH decreases (5, 11, 16). Since renal insufficiency as measured by ¹²⁵I-iothalamate clearance was identical in patients with interstitial nephritis and in patients with glomerulonephritis without the nephrotic syndrome, one must accept that any effect of azotemic serum must be identical in the two groups. As a whole it is questionable whether ¹²⁵I-hippuran and PAH clearances are expressions of "effective" renal plasma flow or of

Table IV. Kidney function studies in patients with chronic glomerulonephritis

Abbreviations as in Table I

Pat. no.	Sex	Age (yr)	C	C _{cr}	C _{cr} /C _{in}	C _{125I-PAH}	FF C _{cr} /C _{125I-PAH}	FF C _{cr} /C _{125I-PAH}	Proteinuria (g/24 h)	S-albumin (g/100 ml)	Hct (%)	RF
32	♂	38	11.1	18.0	2.05	38	23.1	47.4	4.4	3.5	31	175/120
33	♀	56	9.5	16.7	1.76	41	23.2	40.7	1.5	3.6	28	120/90
34	♀	34	10.4	18.2	1.75	36	28.9	50.6	2.9	—	28	190/125
35	♂	50	13.5	23.8	1.76	98	13.8	4.3	2.5	3.4	44	160/110
36	♂	25	14.0	25.5	1.83	46	30.4	55.4	1.5	2.3	30	160/100
37	♀	22	16.4	32.0	1.95	96	17.1	33.3	4.0	2.8	30	160/100
38	♀	26	37.6	50.0	1.33	157	24.0	31.9	0.0	3.2	47	115/85
Average		36	15.7	26.3	1.78	73.1	22.9	40.5	4.0	3.1		
S.D.			10.6	11.7	0.3	45.7	5.9	11.3	4.0	0.5		
S.E.M.			3.8	4.4	0.09	17.3	2.2	4.3	1.5	0.3		

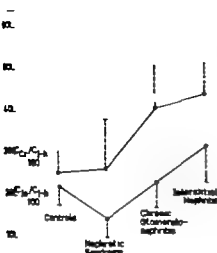


Fig. 3 Relationship between creatinine, ^{125}I -iothalamate and ^{51}Cr -EDTA clearances in controls and patients with kidney diseases.

patients with severe renal insufficiency as self-depression takes place at a lower serum concentration. This self-depression depends partly on the degree of tubular destruction (14, 18).

The low FF values in patients with the nephrotic syndrome could be a result of damage to the glomerular membrane and a low colloid osmotic pressure. Evidence that the low colloid osmotic pressure affects the FF can be seen in Fig. 4, which shows a correlation between concentration of serum albumin and the FF. In two patients with the nephrotic syndrome without glomerulonephritis and excessively low serum albumin, the same low FF was found. The FF level was independent of whether the glomerular lesion was of proliferative or membranous type. A normal or reduced FF value was found in all patients with glomerular lesions. The present investigation did not allow evaluation of which of the three factors—glomerular lesion, tubular lesion, or changes in the colloid osmotic pressure—had the greatest influence on the functional pattern.

In this study BP had only a slight influence on the FF level. Half of the patients in groups II, III and IV were hypertensive. The mean FF was somewhat higher in the hypertensives but a significant difference was not found.

In the controls there was no significant difference between creatinine and ^{125}I -iothalamate

clearances. In groups II, III and IV creatinine clearance was significantly higher than ^{125}I -iothalamate clearance in all cases. In patients with the nephrotic syndrome the creatinine clearance values were on an average 85% higher than the ^{125}I -iothalamate clearance values which means that 42% of the amount of creatinine excreted was eliminated by a mechanism other than glomerular filtration.

Thus under pathological conditions creatinine does not meet requirements for a substance which alone is excreted by glomerular filtration (2, 1, 20). In patients in terminal uremia there is no longer any relationship between the nature of the renal disease and the level of the FF.

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LACTOFERRIN AND IRON ABSORPTION IN THE SMALL INTESTINE

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Abstract. Lactoferrin (LF), an iron-binding protein, has been studied in (1) duodenal fluid of 16 hematologically normal patients, (2) duodenal fluid of 9 patients with iron overload (idiopathic and secondary hemosiderosis), (3) T-drain bile of 14 cholecystectomized patients. Patients with iron overload show on an average lower LF values in the duodenal fluid than normal individuals. This relation with iron metabolism is also present in the group of normal controls. Higher iron and iron saturation levels of the plasma are accompanied by lower LF values. In the first days after cholecystectomy this relation is lost. Purified human LF brought into the duodenum, distinctly inhibits iron absorption in the intestine during the first hours of absorption. However this effect is absent in patients with idiopathic hemosiderosis.

Lactoferrin (LF) is an iron-binding protein, originally discovered in cow-milk by Sørensen and Sørensen (28) but later also found in other external secretions such as bile, pancreatic fluid and small intestinal secretions of many species including man (3, 4, 10, 12, 17, 18, 19, 20, 26, 27).

LF has much resemblance to transferrin (TF). Both proteins are composed of a single polypeptide chain (1, 7), have a nearly identical molecular weight of approximately 80 000 and can bind metal atoms to two specific metal-binding sites of each molecule.

Human LF is saturated with iron at a metal:protein ratio of about 0.025 μ moles/mg protein (20) but in its native form in milk LF was found to be saturated with iron to about 20% of its iron-binding capacity (17). The LF-iron complex is very stable between pH 4 and 11. Between pH 4 and 2 the complex is even more stable than the TF-iron complex (17).

Human LF has a pronounced tendency to form complexes with more acid proteins for instance

casein or albumin (11). In vitro it could be proved (11) that these interactions influence the electrophoretic behaviour of LF. Electrophoresis of the isolated protein in agarose reveals migration to the γ -region. In contrast to the naturally occurring LFs which show a line of precipitation in the β -region.

Though there are many similarities between LF and TF the two proteins show distinct differences when one studies their antigenic properties, amino acid compositions, carbohydrate moieties and tryptic peptide patterns (4, 20, 21). Furthermore LF is normally not detectable or only in very low concentrations in blood plasma by radioimmunoassay (25).

For LF as well as for TF two possible functions can be considered. (1) Bacteriostatic function. LF not saturated with iron shows in vitro a bacteriostatic effect that is abolished if LF is saturated with iron (6, 17, 18, 19, 20, 27). (2) One can consider the possibility of role of LF in iron transport in the intestine because LF is found in considerable quantities in gallbladder bile and small intestinal fluid.

There are only a very few studies on the influence of LF in bile, pancreatic fluid and small intestinal secretions on iron absorption (5, 13). We therefore studied in more detail LF concentrations in bile of patients with a choledochus drain after cholecystectomy and also compared LF concentrations in duodenal fluid of hematologically normal patients and patients with idiopathic or secondary hemosiderosis. We investigated the relations between LF and some parameters of iron metabolism. Furthermore we studied the influence of intraduodenally applied purified LF on the first hours of iron absorption.



Fig. 1 Immunodiffusion in agar. (a) Upper well = human milk, left bottom well = rabbit antiserum against human LF, right bottom well = rabbit antiserum against human TF. In human milk LF as well as TF are present. The precipitation lines show a picture of non-identity.

(b) Upper well = human ferritin, bottom well = human LF. Left well = rabbit antiserum against human ferritin, right well = rabbit antiserum against human LF. No cross-reactions are observed.

MATERIAL AND METHODS

Isolation of lactoferrin from human milk

LF was prepared from human milk by centrifugation at 5000 g for 30 min to separate the fat from the milk and afterwards at 20 000 g for 1 hour at 4°C in order to remove casein.

The supernatant is saturated with iron by addition of 1 ml $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution (containing 1 mg Fe^{++}/ml) in every 100 ml. After standing for 4 hours a red colour develops. Most of the proteins of the supernatant precipitated by stepwise taking out with ammonium sulphate at pH 8.0 and removed by centrifugation at 5000 g for 1 hour. LF remains in the supernatant till ammonium sulphate concentration has reached 45%. The remaining proteins including LF precipitate.

After dissolving, the precipitated crude product is dialysed against running tapwater for 24 hours and afterwards applied to a column of DEAE-cellulose equilibrated with a phosphate buffer pH 7.4. LF was eluted by the same buffer using stepwise increased concentrations from 0.005 M to 0.05 M. After pooling, the fractions containing LF were applied to a Sephadex G-200 column, equilibrated with Tris-HCl 0.1 M in 1 M NaCl, pH 8.0. LF free of detectable contaminants, was eluted by means of the same Tris-HCl buffer.

On an average 100 mg pure LF was prepared from 100 ml milk. This isolated salmon-pink protein contains 0.11% iron. It can bind radioactive iron as could be proved by autoradiography.

Immunoelectrophoresis using rabbit antiserum against human serum proteins (Nordic Pharmaceutical and Diagnostics, Tübing, The Netherlands) did not reveal any serum contaminants in our LF samples.

Preparation of antiserum

Antiserum against LF was prepared in rabbits by four i.m. injections of 0.5 mg LF emulsified in 0.5 ml phos-

phate buffer pH 7.4 administered at intervals of 14 days. On the first two occasions complete Freund's adjuvant was added to the emulsion. The antiserum mostly revealed only one precipitation line with LF but sometimes with human milk one or two faint extra precipitation lines appeared.

After absorption with pooled human serum these contaminants were no longer detectable. Especially, no cross reactions with TF and ferritin (Fig. 1 and 2).

Antiserum against human TF (Nordic Pharmaceutical and Diagnostics, Tübing, The Netherlands) was prepared in a rabbit in the same way as antiserum against LF. After absorption of this antiserum with albumin and acid serumoid fraction only one precipitation line with human TF and human serum was obtained.

Quantitative determination of lactoferrin, transferrin and IgG

In bile and duodenal fluid LF, TF and IgG were determined by the single diffusion test (15, 16). For quantitative determination of IgG we used commercially available agar-gel plates with specific IgG antiserum (Pargen Immunodiffusion plates, Behringwerke Marburg/Lahn, W. Germany).

Iron determination in serum, bile and duodenal fluid

The serum iron concentration and total iron-binding capacity (TIBC) were measured by colorimetric determination with bathophenanthroline disulphonate in deproteinized plasma (24, 29). The iron concentration in bile and duodenal fluid was measured after wet ashing (14) by colorimetric determination as above (24, 29). In both determinations we used the commercially available iron test combination of Boehringer Mannheim, W. Germany.

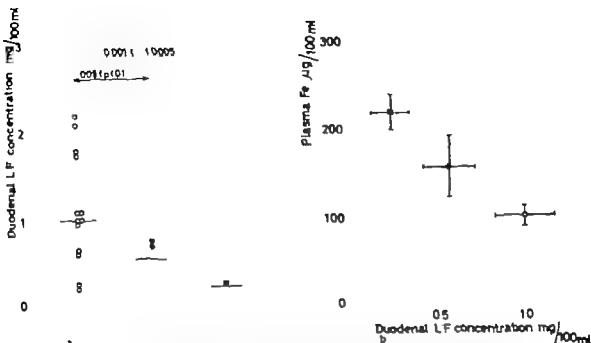


Fig. 2. (a) LF concentration in non-stimulated duodenal fluid. ○=16 controls (mean LF concentration 1.0 mg/100 ml), ●=5 patients with idiopathic hemochromatosis (mean LF concentration 0.56 mg/100 ml), ■=4 patients

with transfusion hemochromatosis (mean LF concentration 0.23 mg/100 ml). (b) Plasma iron and duodenal LF concentration of the three groups of patients (mean \pm S.E.M.).

Determination of radioactivity

Radioactive iron ($^{59}\text{Fe}^{++}$ -ascorbate and $^{59}\text{Fe}^{++}$ -citrate) was obtained from the Radiochemical Centre, Amersham, UK. The radioactivity of ^{59}Fe in plasma was counted with sodium iodide well-type crystal. The ^{59}Fe plasma samples were mixed with suitable amounts of Insta-gel (Packard) and counted in a liquid scintillation counter.

Determination of iron absorption in the whole-body counter

Absorption and retention of ^{59}Fe in two patients with idiopathic hemochromatosis were also measured in the whole-body counter of the Radiological Department, Health Organization, Bilthoven, The Netherlands.

Selection of patients

The following groups of patients were studied:

I. In 14 patients we continuously sampled bile delivered by a T-drain after cholecystectomy. All patients had normal liver functions. None was transfused. No blood transfusions were required during or after operation. The following measurements were made: Blood Hb concentration (g/100 ml), Hct (%), serum iron concentration ($\mu\text{g}/100\text{ ml}$), TIBC and latent iron-binding capacity (LIBC) ($\mu\text{g}/100\text{ ml}$), iron saturation (%), *gld* LF (mg/100 ml and mg/24 hours), TF (mg/100 ml and mg/24 hours) IgG (mg/100 ml and mg/24 hours).

II. Sixteen hematologically normal patients were se-

lected for measurement of LF concentrations in non-stimulated duodenal fluid obtained by duodenal intubation. These patients had normal liver function tests and no pancreatic or gallbladder disease.

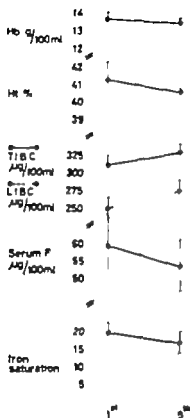
III. The same study was performed in 9 patients with iron overload. In 3 of them idiopathic hemochromatosis was present as proved by liver biopsy, desferrioxamine test, family history and other clinical parameters. In 4 patients a secondary hemochromatosis due to blood transfusions during chronic hemodialysis existed.

IV. In 11 hematologically normal controls (not the same individuals as studied in group II) and in 3 patients with an idiopathic hemochromatosis (already mentioned in group III) we studied the effect of purified human LF on the initial plasma radioactivity after duodenal administration of two tracer doses of radioactive iron: one with and one without 25 mg LF.

As human gallbladder contains on an average 10–30 mg LF a quantity of 25 mg, as used in our experiments can be considered physiological one.

The composition of the test solution was: 8 μCi $^{59}\text{Fe}^{++}$ -citrate or $^{59}\text{Fe}^{++}$ -ascorbate with 10 μmoles Fe^{++} as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ as carrier and 5 ml 0.1 M citric buffer pH 6.2. This solution was brought directly through tube into the duodenum in order to prevent deactivation of LF by gastric juice. We administered LF alternately with the first and the second tracer active iron in order to avoid systematic error of instrumentation. The twofold

BLOOD



BILE

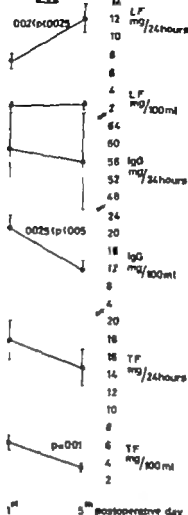


Fig. 3 Comparison of data from the 1st and 5th postoperative days in 14 cholecystectomized patients (mean values)

active tracer doses to the same patient once with and once without LF eliminates individual differences in iron absorption.

Statistic methods

Differences in iron concentration, radioactivity and LF concentration were tested for statistical significance by the Student *t*-test (8).

RESULTS

1 Lactoferrin in Non-stimulated Duodenal Fluid

Studying the relation between iron metabolism and LF we compared the LF concentration in non-stimulated duodenal fluid of 16 hematologically normal control patients, 5 patients with an idiopathic hemosiderosis and 4 with a secondary hemosiderosis. All patients with idiopathic and

secondary hemosiderosis had a LF concentration in the duodenal fluid lower than 1 mg/100 ml.

In the group of hematologically normal control patients a great variability of the LF concentration in the duodenal fluid exists, but the difference in mean value between the normal and iron-loaded patients is highly significant ($p < 0.01$). Our findings are shown in Fig. 3.

Considering the difference in LF concentration in normal individuals and patients with iron overload we studied in the group of normal controls the relation between LF and some parameters of iron metabolism. The high variability of LF in normals proved also to be related to the iron status. High plasma iron and iron saturation proved to be associated with low LF values (plasma iron - duodenal LF $r = -0.57$, $p = 0.01$, $n = 16$; iron saturation - duodenal LF $r = -0.63$, $p = 0.005$, $n =$

16). A higher TIBC is accompanied by a higher LF concentration ($r=+0.51$ $0.01 < p < 0.025$ $n=16$).

Thus these findings point in the same direction as our findings in patients with iron overload though in the latter a more extreme situation exists. This is illustrated in Fig. 2b.

II Lactoferrin Concentration in Bile after Cholecystectomy

To study the relation between iron metabolism and bile LF we also investigated 14 patients during the first 5 postoperative days after cholecystectomy.

The mean values of the results on the first and fifth postoperative days were compared and are illustrated in Fig. 3. We chose these days because a fairly steady state exists from the fourth postoperative day and oral iron uptake is negligible up to the sixth day. Also excretion of IgG and TF in the bile are constant from the fourth postoperative day (Fig. 4).

As expected the mean values of Hb concentration, Hct and serum iron show a slight decrease postoperatively while TIBC and LIBC increase. These changes however are not significant. At the same time the 24-hour LF excretion in bile increases significantly whereas the concentrations and the 24-hour excretions of TF and IgG in bile decline. A typical example of our findings is presented in Fig. 4.

From Figs. 3 and 4 it may be concluded that in the first days some plasma leakage occurs in the bile ducts, very probably caused by surgical manipulation and slight inflammatory reaction, resulting in biliary TF and IgG loss and diminishing on the next postoperative days. LF however shows a rising biliary excretion during these days, thus demonstrating an opposite effect to TF. Apparently the two proteins in bile behave differently (10).

Also in this group of patients we analysed the relation between biliary LF and some parameters of iron metabolism. The correlations (r) are presented in Table I. From Table I we can conclude that the inverted relation between iron content and LF found in the duodenal fluid of normals and patients with iron overload is lost in cholecystectomized patients.

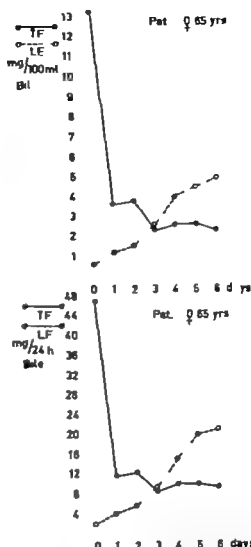


Fig. 4 LF and TF in bile of an individual patient after cholecystectomy. Top: LF and TF concentration. Bottom: 24-hour LF and TF excretion of the same patient.

III Effect of Lactoferrin on Iron Absorption

(a) Hematologically normal control patients

The laboratory data of 11 hematologically normal control patients (group IV) are summarized in Table II.

In all patients of this group except one the plasma radioactivity 1, 2 and 3 hours after the test dose of radioactive iron is lowered by administration of 25 mg LF into the duodenum (Fig. 5).

Plasma radioactivity is expressed as per

Table 1 Correlation between LF in bile and plasma iron, iron saturation, Hb concentration and Hct on the first and fifth postoperative days

Blood	LF in bile			
	1st day		5th day	
	mg/100 ml	mg/24 h	mg/100 ml	mg/24 h
Hb (g/100 ml)	+0.72	+0.68	+0.60	+0.63
Hct (%)	+0.45	+0.48	+0.51	+0.63
Fe (μ g/100 ml)	+0.72	+0.60	n.s.	n.s.
Fe saturation (%)	+0.62	+0.57	n.s.	n.s.

Only significant correlations ($p < 0.05$) are presented. n.s. = non-significant.

centage of the oral dose of radioactive iron. The inhibitory effect of LF on the plasma radioactivity can be calculated by the ratio of the plasma radioactivity after an oral dose of radioactive iron with and without LF. This ratio is expressed as $[\text{Fe}(+\text{LF})/\text{Fe}(-\text{LF})] \times 100\%$. The average plasma radioactivity measured 1, 2, and 3 hours after the test dose of 25 mg LF is considerably diminished to respectively 79.5%, 41.4% and 53.4% of plasma radioactivity after the same test dose without LF (Table III Fig. 6).

One patient (case 9) suffering from a chronic gastritis, reacted completely differently from the other patients for reasons yet unknown. Careful clinical investigation revealed no other abnormalities. Liver biopsy was refused, so the iron content

of the liver remained unknown. Control experiments performed with 25 mg albumin ($n=3$), 25 mg TF ($n=3$) and 25 mg IgG ($n=3$) showed no inhibitory effect on the plasma radioactivity curve.

(b) Patients with idiopathic hemosiderosis

The same investigation was made in 5 patients with idiopathic hemosiderosis. The hematological data from these 5 patients are summarized in Table II. All patients showed a positive desferrioxamine test and iron accumulation in liver biopsy. As expected there is a large variability in the hematological data of this group because some patients had been treated by phlebotomy very recently.

There is no distinct inhibitory effect of LF on

II Hematological data from normal controls and patients with idiopathic hemosiderosis

	Sex	Age (y)	Fe (μ g/100 ml)	TIBC (μ g/100 ml)	Saturation	Hb (g/100 ml)	Hct (%)	Last phlebotomy
<i>Normal controls</i>								
1	♂	48	114	299	36	14.8	45	
2	♂	22	94	297	29	14.2	44	
3	♂	38	85	284	30	14.3	44	
4	♂	42	76	270	28	14.4	42	
5	♂	48	137	285	47	17.8	53	
6	♀	74	109	313	34	13.8	42	
7	♂	30	115	370	32	14.4	44	
8	♂	30	110	320	34	14.7	45	
9	♀	41	88	366	23	14.2	41	
10	♂	24	184	297	64	16.8	51	
11	♂	45	150	312	48	14.9	46	
<i>Patients with idiopathic hemosiderosis</i>								
1	♂	48	200	280	71	16.8	50	1 y
2	♂	46	43	276	16	14.5	44	1 y
3	♂	62	120	282	47	16.0	50	5 d
4	♀	45	225	40	97	14.8	46	14 d
5	♂	57	10	340	60	15.5	48	1 mo

All patients with idiopathic hemosiderosis showed positive liver biopsy with iron accumulation and a positive desferrioxamine test.

the plasma radioactivity curve (Table III Fig. 7). The difference between the controls and the patients with idiopathic hemosiderosis with respect to the effect of LF on the plasma radioactivity in the first hours after a test dose of radioactive iron is highly significant. After 3 hours there is a non-significant difference (Fig. 6).

Two of these patients were investigated with whole-body counting 10 and 14 days after the test dose of ^{59}Fe with and without LF. Both patients had as expected a very high iron retention after 10 and 14 days. Here we found no significant difference between the iron retention after a test dose with and without LF (Fig. 7).

DISCUSSION

Very few data are available in the literature about the relation between the iron-binding protein lactoferrin and iron absorption in the small intestine.

Blanc et al. (5) determined the influence of LF on intestinal iron absorption in two patients, in one of whom they found a significant decrease in absorption. However a slight increase was shown in the red cell uptake of radioactive iron in both patients after 10 days. De Laey et al. (13) studied the effect of human LF on iron absorption in rats using everted duodenal sacs (16). They concluded that exogenous apo-lactoferrin (iron-deprived protein) significantly decreased the mucosal iron uptake but iron-saturated LF did not much influence iron uptake in the mucosal cell. The accumulation of iron in the duodenal mucosa of guinea pigs was significantly higher after pre-incubation of duodenal loops with LF antibodies. From these experiments they concluded that LF is of importance for the control of iron absorption in the small intestine.

Our results also suggest a relation between LF in bile and duodenal fluid and iron metabolism in man. In normal individuals higher values for plasma iron and plasma-iron saturation are associated with lower duodenal LF concentration and vice versa. In patients with iron overload in idiopathic hemosiderosis as well as in secondary hemosiderosis, the duodenal LF is low representing a more extreme example of the relations already found in hematologically normal individuals.

Now the question arises whether higher iron saturation of the body especially the liver causes

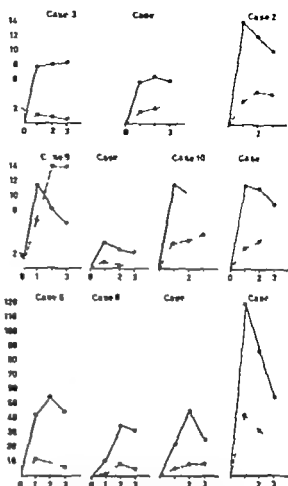


Fig. 5 Plasma radioactivity curves of 11 normal control patients. Plasma radioactivity after an oral tracer dose of 25 mg LF is always lower than after the same tracer dose without lactoferrin, except in one patient (no. 9). —radioactivity/100 ml plasma (percentage of the oral tracer dose 10^{-6}). —time (h), ○—○— ^{59}Fe without LF ○—○— ^{59}Fe with LF.

the low LF excretion in the bile or whether the causal relation is the reverse in the sense that LF poured out into the duodenum, inhibits iron absorption. Our studies with purified LF brought into the duodenum are very suggestive of the latter view. The inhibition of iron absorption is very significant in the first hours of iron absorption. The LF dose we used is a physiological one. The gall bladder contains ± 10 –30 mg LF and each contraction of the bladder pours out such a quantity of LF into the duodenal lumen.

We realize that the plasma radioactivity after iron isotope administration is only an indirect

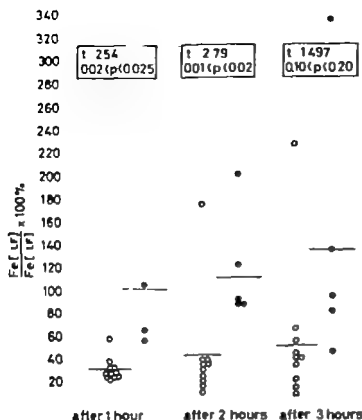


Fig 6 Comparison of the inhibitory effect of L.F. on the plasma radioactivity after an oral tracer dose of radioactive iron in normal controls (O) and patient with idiopathic hemosiderosis (●). After 1 and 2 hours a statistically significant difference between the two groups is observed. After 3 hours there is no significant difference.

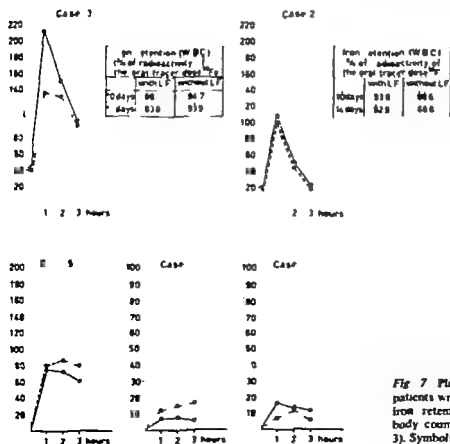


Fig 7 Plasma radioactivity curves of 3 patients with idiopathic hemochromatosis and iron retention determination by whole-body counting in 2 patients (nos. 2 and 3). Symbols as in Fig. 5

Table III Inhibitory effect of lactoferrin on plasma radioactivity in normal controls and patients with idiopathic hemosiderosis

Pat. no.	1 hour (%)	2 hours (%)	3 hours (%)
Normal controls			
1	36	35	4
2	21	37	40
3	26	10	6
4	26	15	40
5	30	30	53
6	26	-	1
7	23	18	33
8	4	23	16
9	57	173	226
10	30	38	-
11	25	35	66
Mean	29.5	41.4	53.4
Patients with idiopathic hemosiderosis			
1	40	85	44
2	91	88	80
3	64	96	95
4	201	200	333
5	104	120	139
Mean	100.0	115.8	136.6

measure of the initial iron absorption and that the inhibition we found does not mean that ultimate iron retention is also diminished. Further studies will be necessary to elucidate the influence of LF on iron absorption in the lower parts of the intestine. LF cannot in our view exert its inhibitory effect on initial iron absorption by molecular binding of iron ions because we used nearly iron-saturated LF (80%).

As yet one can only speculate about the mechanism of this inhibitory action. The special affinity of LF for other proteins may be of importance to this phenomenon. LF is a peculiarly sticky protein and one can consider the possibility that it blocks mucosal iron receptors of a protein nature.

A very interesting point in connection with this hypothesis is our finding that in primary hemosiderosis this blocking effect of LF is absent. These patients have a very low LF concentration in their duodenal fluid but its blocking effect is lost too. Whether this is an essential feature of primary hemochromatosis we cannot say. It could be a result of iron accumulation in the mucosa. Studies in secondary hemochromatosis could help to solve this problem, but unfortunately the absorption in these cases is so low that further in-

hibition by LF cannot be clearly demonstrated. But perhaps biliary LF is a protective system against too high iron absorption which is defective in primary hemochromatosis.

Our studies of biliary LF in cholecystectomized patients also require some discussion. Here the normal relations between some parameters of iron metabolism and lactoferrin are lost. Shortly after operation plasma iron, iron saturation, Hb and Hct are positively correlated with biliary LF concentration. Instead of the negative correlation we found in normal individuals.

On the other hand the mean value of the 4-hour biliary LF excretion in the cholecystectomized group shows a significant rise during the next postoperative days together with a non-significant fall of Hb, iron concentration and iron saturation of the plasma, suggesting again the same relation as we found in normal individuals.

An explanation of all postoperative findings is hampered by the fact that surgical intervention and stress strongly influence plasma iron and TF which very probably are trapped in the RES (22, 23). Other complicating factors are blood loss, release of iron from hematomata combined with very reduced oral iron uptake, loss of iron and LF through the T-tube and postoperative variations in bile flow. Another disturbing factor may be slight liver dysfunction due to anaesthesia. Furthermore biliary LF values before operation are unknown.

We think, therefore, that cholecystectomized patients are not a suitable model for studying exactly the relations between biliary lactoferrin and iron metabolism.

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ITCHING IN PREGNANCY

A 15-year Follow-up Study

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Abstract All pregnancies in 89 women who had had itching during pregnancy have been characterized on the basis of personal interview and available hospital records. Most of the non-cholecystectomized women underwent an oral cholecystography. The majority of the women were between 35 and 55 years old. The study was performed approximately 15 years after their last complicated pregnancy. Itching had recurred during subsequent pregnancies in 34 of the 71 women with more than one full-term pregnancy. Delivery tended to occur earlier in pregnancies with itching (median 40th week) than in uncomplicated ones (median 41st week). Children born at deliveries with itching were somewhat lighter than those born after uncomplicated pregnancies (median 3365 and 3500 g). Meconium staining of the amniotic fluid was observed in 27% of deliveries with itching and in 9% of those without. Four children born to women with recurrent itching died from asphyxia at or shortly after birth. Two of the pregnancies had been complicated by itching. Evidence of earlier (cholecystectomy) or present (abnormal cholecystography) cholelithiasis was found in 35 women. The minimal prevalence was higher in women with recurrent itching (59%) than in the remainder (27%). The combined evidence from this and previous study indicates that the minimal prevalence of cholelithiasis in women 35-60 years old with history of recurrent cholestasis of pregnancy is 58%. The figure is significantly higher ($p < 0.001$) than that encountered in women of comparable age in two Swedish autopsy series.

Cholestasis of pregnancy is a syndrome characterized by itching with or without jaundice. It usually appears in the last trimester and the symptoms disappear at or shortly after delivery (12). Several reports demonstrate that cholestasis of pregnancy is associated with a slightly shortened gestation (4, 5, 8, 12, 13, 19, 20, 22, 30, 31), a subnormal birth weight of the child (9, 24, 25, 30) and a high incidence of fetal

25). The cause of cholestasis of pregnancy is not known. A recent study demonstrated a familial predisposition in women with recurrent symptoms during several pregnancies (10). These women furthermore developed gallbladder disease to an unexpectedly high extent (11).

The reports cited above concern women with cholestatic jaundice of pregnancy. The milder and probably most common form of cholestasis of pregnancy (26) is characterized by itching in the absence of jaundice (1). The object of the present study was to investigate whether the observations referred to above are valid for women with pruritus during pregnancy as the dominant symptom.

MATERIAL AND METHODS

From March 1953 to Feb 1956 Arfwedson, who had been the first to prove the association between generalized itching of pregnancy and liver dysfunction (2), investigated 100 women with itching during pregnancy randomly selected among the patients admitted to the Department of Obstetrics of Malmö General Hospital in the south of Sweden. With the permission of Dr Arfwedson and with access to the names, birth dates and hospital record numbers of the women, 98 of them could be traced through population registers.

The present study included an interview by telephone, following standard questionnaire. Further information was obtained from hospital records. Most deliveries (216 of 49) took place at Malmö General Hospital. During Arfwedson's investigation notes were made in the delivery records of the presence or absence of itching and jaundice during the current and previous pregnancies. In delivery records written after Arfwedson's investigation the presence of itching was usually noted as complication of pregnancy. The recollections of the women regarding the presence or absence of itching and jaundice usually

Table 1 Number of pregnancies in relation to recurrence of itching

Group	No of women	Full-term pregnancies		Abortions		Total	
		With itching	Without itching	With itching	Without itching	With itching	Without itching
1	34	108	13	0	18	108	31
	37	36	74	1	16	37	90
3	18	18		0	8	18	8
Total	89	162	87	1	42	163	129

the delivery records but in some cases there was difference concerning the duration of these symptoms. When record were missing, the interviews were sometimes the only source.

All information regarding complications during pregnancy other than itching and jaundice, the date of the last normal menstruation, maternal and fetal complications during delivery and the birth weights of the children, were obtained from delivery records. Other data related below originate from the interviews. The lengths of pregnancies were calculated from the last normal menstruation. The severity of itching and jaundice was roughly graded as described previously (8).

Non-cholecystectomized women were referred to their local hospital for a cholecystography unless such an examination had been performed within the last 5 years.

The χ^2 -test was used in the statistical calculation.

RESULTS

Eighty-nine of the initial 100 women took part in the study. Two women had died (one from carcinoma of the breast, the other in an accident).

Others could not be traced and 7 were unwilling to participate.

The women were divided into 3 groups according to the recurrence of itching during pregnancy.

Table 2 Age (I) of the women at the time of the first pregnancy (I); at the last pregnancy (II) itching (II) and at the follow up study (III) (mean and range)

Group	N of women	I	II	III
1	34	23 17-35	37.1 22-42	44.3 34-59
2	37	23.5 18-31	28.1 18-40	45.1 35-58
3	18	27.4 20-37		44.4 37-54

Group 1 comprised 34 women who had had the symptom during more than one pregnancy. Group 2 included 37 women with itching during one of several full-term pregnancies. The 18 women in group 3 had had only one full-term pregnancy. There were 79 pregnancies in all 43 of which ended in abortion (Table 1). One abortive pregnancy (group 2) was complicated by itching.

The mean age of the women at their last pregnancy complicated by itching was about 30 years. The follow-up study was performed approximately 15 years later (Table 2).

The pregnancies

Group 1 Of the 11 full-term pregnancies of the 34 women, 13 seem to have been free from pruritus (Table 1). Itching always ended at or within a few weeks after delivery. The median duration was about 2 months (range 0.5-7.5). In 20 pregnancies of 11 women there had been visible jaundice in association with itching. The duration of symptoms was somewhat longer in these pregnancies than in those with itching alone (2.5 and 1.5 months respectively). Jaundice was observed about 1 month (median value) after itching had started and ended at delivery. Pregnancies not complicated by itching occurred before, after or between pregnancies with itching, in no discernible pattern.

Group 2 The 37 women underwent 110 full-term pregnancies. The median duration of itching was 1 month (range 1 week - 7 months). Itching usually ended at or shortly after delivery but in 6 pregnancies it disappeared 0.5-3.5 months (median 1.5) before delivery. Itching occurred together with jaundice in 2 pregnancies.

Group 3 None of the 18 women had jaundice during pregnancy. The median duration of itching was 2 months (range 1 week - 4 months). Itching disappeared before delivery in pregnancies

Table III. Length of pregnancies (median and range)

No. of deliveries ending before the 37th week within parentheses

Group	No. of women	Itching at delivery		Itching ending before delivery		No itching	
		No.	Week of delivery	No.	Week of delivery	No.	Week of delivery
1	34	23 (10)	40 31-46			9 (3)	39 26-42
2	37	23 (2)	41 34-43	5 (1)	40 36-41	65 (2)	41 35-45
3	18	16 (4)	40 31-44	2	38-39		
Total	89	127 (16)	40	7 (1)	40	74 (5)	41

(2.5 and 4.5 months) In all other instances it vanished at or shortly after delivery

Hypertension (diastolic BP ≥ 95 mmHg) and/or proteinuria had been found in 25 women during 33 pregnancies. The incidence of proteinuria and/or hypertension was approximately the same in all groups, and was not associated with the presence of pruritus.

There is no indication that any of the women had been exposed to infectious hepatitis prior to a period of itching. Urinary tract infection was observed in 11 women during 12 pregnancies, 9 of which were also complicated by itching. Five women were referred to group 1, 5 to group 2 and 1 to group 3. Four women (group 2) were operated on for appendicitis while pregnant. Two of them developed itching, in one case with jaundice a few days after the operation. The other 2 had no itching during these pregnancies.

The deliveries

Records could be traced from 243 of the 249 deliveries. There were 4 twin deliveries (1 in group 1, 3 in group 2) which are excluded from the following account. All twin pregnancies were complicated by itching up to delivery.

The date of the last normal menstruation was given in 208 records covering deliveries with a spontaneous onset. Delivery occurred in the 40th week (median value) in the 134 pregnancies complicated by itching, and in the 41st week in the 74 without itching (Table III). There was no obvious difference in this respect between pregnancies with itching ending before delivery as opposed to after. In the 15 women in group 1 who had had jaundice delivery occurred in the

39th week (median value) in complicated pregnancies.

In 22 women in group 2 the complicated pregnancy tended to be shorter than the mean of the women's uncomplicated pregnancies (mean difference 1 week). In individual women with more than one complicated pregnancy (group 1) there was no correlation between the severity of itching (or the presence of jaundice) and the length of pregnancy.

An estimation of the blood loss during delivery was given in 234 records. When uterine-contracting drugs (secale alkaloids or oxytocin preparations) were used, the median blood loss in deliveries with itching was 190 ml and 190 ml in uncomplicated deliveries. In the remaining deliveries the median blood loss was 200 ml whether there had been itching or not. There were no discernible differences between women referred to different groups nor between complicated and uncomplicated deliveries in individual women.

The children

The birth weight was registered for 223 children (twins excluded) born at spontaneous deliveries. Children born at deliveries complicated by itching were somewhat lighter than children born after uncomplicated pregnancies (median weight 3365 and 3500 g respectively) (Table IV).

In individual women with recurrent itching (group 1) there was no correlation between the severity of symptoms in the presence of jaundice and the birth weight of the child. In the 24 women in group 2 in recurrent cases with

Table IV. Birth weights (g) of the children in relation to itching during pregnancy (median and range)

No. of premature children (birth weight < 500 g) within parentheses

Group	No. of women	Itching at delivery		Itching ending before delivery		No itching	
		No.	Birth weight	No.	Birth weight	No.	Birth weight
1	34	93 (7)	3350 340-4560	-		10 (2)	3700 1020-3900
	37	26 (1)	3410 030-4250	5	3260 2900-3680	7 (*)	3530 2200-5330
3	18	15 (2)	3380 1860-4350		3550 3860	-	
Total	89	134 (10)	3365	7	3400	82 (4)	3300

ing during delivery the child born at that delivery tended to have a slightly lower birth weight than the mean of its siblings (mean difference 200 g).

Four children born to 3 women in group 1 died from asphyxia during or shortly after delivery. In all instances the diagnosis was made at autopsy. There had been itching during 2 of the pregnancies, one of which was also complicated by preeclampsia and elevated BP. The mothers of the children born after uncomplicated pregnancies had itching with jaundice during other pregnancies. Gestation varied from 35 to 45 weeks and the birth weights from 220 to 950 g.

Three children (2 from group 1 and 1 from group 3) who died in utero before delivery were born asphyxiated. No specific cause of death could be established. There had been itching during 2 of the pregnancies.

Notes of meconium staining of the amniotic fluid, considered a sign of fetal asphyxia (16) were found in 48 delivery records (Table V) in the absence of obstetric complications or disease of the child. The incidence was 9% (8 of 86) in

children born after uncomplicated pregnancies and 77% (37 of 149) in those born in deliveries with itching. Among the latter there was no difference between children of women with recurrent and non-recurrent symptoms. Factors which might increase the risk of asphyxia (infection during pregnancy, proteinuria, elevated BP, premature delivery or low birth weight) were present in 15 of the 37 deliveries with itching, and in 4 of the 8 deliveries without.

Rickets was discovered at the age of 8 months in one child born after a pregnancy complicated by itching. Several other children had had medical care for reasons other than infectious disease and minor traumata, but there was no concentration of any disease or anomaly to any special group.

The women

Eleven women (12%) reported that a close relative had had itching during pregnancy (Table VI). The highest prevalence (18%) was found among the women with recurrent itching (group 1).

At the follow-up the majority of the women later

Table V. Deliveries with meconium staining of the amniotic fluid

Group	Itching at delivery		Itching ending before delivery		No itching	
	Total	With meconium staining	Total	With meconium staining	Total	With meconium staining
1	101	4			13	1
2	30	7	6	3	73	7
3	18	6	2	0	-	
Total	149	37	8	3	86	8

Table VI. *Itching and jaundice during pregnancy in relatives of the women investigated*

Group	Case no.	
1	17	Mother had itching during all 8 pregnancies
	21	Mother had itching during her only pregnancy
	26	Sister had severe itching during 1 of 2 pregnancies
	37	Sister had itching during her only pregnancy
	79	Sister had itching during her only pregnancy
2	96	Daughter had itching during her only pregnancy
	13	One of 2 sisters had itching during all 3 pregnancies
	47	Sister's daughter had itching during pregnancy
	80	Daughter of maternal uncle had itching during her only pregnancy
3	96	Maternal aunt had jaundice during 1 of 5 pregnancies
	32	Mother had itching during the first of 4 pregnancies
	94	Mother had itching during one or more of 3 pregnancies

viewed considered themselves to be in good health. Of the 89 women 69 have been admitted to hospital for reasons other than childbirth and complications during pregnancy. Apart from biliary tract disease the most common causes of admission have been benign disorders requiring surgical or gynecological treatment. Medical care for urinary tract disease was reported by 11 women, 3 of whom (1 from each group) had suffered from recurrent urinary tract infection.

Nineteen women had been cholecystectomized because of gallstones, and 16 showed gallstones at cholecystography giving a minimal prevalence of cholelithiasis of 39% (Table VII). Twenty-one women (7 in group 1, 21% 10 in group 2, 27%

4 in group 3, 22%) did not consent to a cholecystography. The prevalence of present or previous cholelithiasis was higher among women with recurrent than non-recurrent itching (59 and 24% respectively). The prevalence was 41% (28 of 68) in women below 50 years of age and 33% (7 of 21) in those above that age. Of the women who were unwilling to undergo X-ray examination 5 (3 in group 1) had symptoms suggesting gallbladder disease. These women had experienced epigastric or upper right abdominal pain following ingestion of fats, eggs or raw fruits. One woman (group 2) was cholecystectomized 3 years before her period of itching and 4 women (group 1) were operated on between pregnancies with itching. The remaining 14 were cholecystectomized 6-17 years (median 12) after the first period of itching.

Twenty-seven women had tried oral contraceptives for periods ranging from a few months to 8 years (median 2.5 years). Ten of them were referred to group 1. None had experienced itching or jaundice during these periods.

DISCUSSION

Cholestasis of pregnancy may be difficult to differentiate from other conditions associated with itching and jaundice such as infectious hepatitis. Laboratory examinations including determination of the Au antigen and liver biopsy facilitate the diagnosis. In the present women the diagnosis of cholestasis of pregnancy was made mainly from the clinical picture. The recurrence of typical symptoms during successive pregnancies in the women of group 1 strongly indicates that they suffered from cholestasis of pregnancy. Retrospectively this diagnosis may be questioned in some women with non-recurrent symptoms. Thus it is remarkable for instance, that itching ended before delivery in 8 women in group 2. In the

Table VII. *Prevalence of gallstones*

Group	No. of women	Cholecystectomized or abnormal cholecystography	Normal cholecystography	Cholecystography not performed	
				Symptoms of gallstones (Typical)	(Non-typical)
1	34	20	7	3	4
2	37	9	16	2	8
3	18	6	8	0	4
Total	89	35	31	5	16

remainder the clinical course was entirely compatible with cholestasis of pregnancy. When only one of several pregnancies is complicated by itching the question of precipitating factors arises. In the present series the only factor found to be over-represented in pregnancies with non-recurrent itching was twin births. Twins were born after 3 of the 36 full-term pregnancies complicated by itching in group I. An overrepresentation of twin pregnancies in cholestasis of pregnancy has been observed earlier (13, 19). It is conceivable that a larger hormone load in such pregnancies may be of importance.

Infections, especially bacterial urinary tract infections, have been implicated as possible provoking and aggravating factors in jaundice of pregnancy (11, 13, 24, 27, 28, 31). However such disease was not common among the present women. Urinary tract infection was diagnosed during 1 pregnancy of which 9 were complicated by itching. It is usually not clear from the records whether itching occurred in temporal association with the infection. Furthermore when the women were not pregnant they do not seem to have suffered from urinary tract disease unduly frequently as far as can be ascertained from the interviews.

Disregarding the possibility that itching may have been caused by disease other than cholestasis in a few women the incidence of cholestasis may be calculated. During the time when the present series (usually 100 women) was collected there were approximately 3000 deliveries at Malmö General Hospital giving an approximate incidence of cholestasis of pregnancy of 3%. In two recent Swedish investigations (14, 23) the corresponding figures are 1% and 4%. The incidence of cholestatic itching is reported to vary from 0.27 to 14% in other countries (7, 15, 26). The last figure originates from Chile, thus corroborating the impression that cholestasis of pregnancy has a peculiar geographical distribution being most often reported from Chile and the Scandinavian countries (1).

Among the present women delivery tended to occur earlier in pregnancies with itching (40th week) than in uncomplicated pregnancies (41st week). The earliest median time for delivery (39th week) was found in complicated pregnancies of those women who had jaundice. This is in accordance with the figures from complicated deliveries

in the previous study of women with jaundice in pregnancy (8). An unexpectedly short gestation has been observed in some cases of itching without jaundice (3, 32) but in a recent Swedish report (14) the time of delivery in 57 women with cholestatic itching was considered normal (median 40th week).

Other reports of women with itching due to cholestasis of pregnancy (6, 14, 18, 26) indicate that the children both of patients and normal controls have approximately the same birth weight. In the present series children born after pregnancies with itching tended to have a lower median birth weight than those born after uncomplicated pregnancies comparable with the shorter gestation period in the former.

Meconium staining of the amniotic fluid, considered to be a sign of fetal asphyxia, was found more often if itching had occurred at delivery (77%) than if no itching had occurred during pregnancy (9%). Additional complications known to cause asphyxia do not explain this discrepancy as they were present to an equal extent in pregnancies with and without itching. The present incidence of 77% is in accord with the figure of 22% for meconium staining of the amniotic fluid in a previous follow-up study among children whose mothers had had itching and/or jaundice at delivery (9). Fetal asphyxia was also described in a report of 9 cases of pruritus of pregnancy (3).

Among the present children there is nothing to indicate that mortality from causes other than asphyxia should be related to the mother's itching. The child with rickets is the only one in whom itching during the mother's pregnancy may be suspected to have caused impaired health. The mother had itching from the beginning of the 8th month until after delivery in the 39th week. Two other children in whom rickets was discovered at an early age and whose mothers had jaundice as well as itching when pregnant, have been reported earlier (9, 21). It has been suggested that malabsorption of vitamin D in the mother due to cholestasis could predispose to a deficiency state in the child (9).

The high prevalence of cholelithiasis in women with recurrent cholestasis was the most striking finding of the present follow-up. The overall minimal prevalence in the present series was 39% (35 of 89). Although this may seem a high figure the prevalence of gallbladder disease in two recent

Table VIII Prevalence of gallstones (GS) in relation to number of full-term pregnancies and age at follow-up (mean and range)

Group	No. of women with GS*	Age (y)		No. of full-term pregnancies	
		GS	No known GS	GS	N known GS
1	29/34	43.1 35-57	43.9 34-59	3.6 2-7	3.5 2-8
2	9/37	47.8 41-58	44.2 35-56	3.2 2-6	2.9 2-6
3	6/18	43.6 40-47	44.8 37-54	1	1
Total	33/89	44.4	44.8	3.1	2.6
GS/total no					

autopsy studies from Malmö (17-29) in women of comparable age was 35 and 31% respectively. There is, however, a pronounced difference between the women with recurrent itching (group 1) and the other women (groups 2 and 3). In group 1 the minimal prevalence of gallstones was 39% (20 of 34) whereas the corresponding figure for groups 2 and 3 was 27% (15 of 55). As among the women with jaundice in pregnancy it is unlikely that the difference in prevalence of cholelithiasis between the present women with recurrent and non-recurrent itching depends on differences in parity or age (Table VIII).

The prevalence of gallbladder disease among the women with recurrent itching is in good agreement with that (16 of 28) found at a previous follow-up study of women with jaundice due to recurrent cholestasis of pregnancy (11). Combining the two series the minimal prevalence of gallbladder disease is 58% (36 of 62) in women who have suffered recurrent cholestasis of pregnancy. By comparison, when the two autopsy series are combined, the prevalence of gallbladder disease is 33% (104 of 313). This difference is statistically significant ($p < 0.001$).

Most of the women considered themselves to be in good health at the follow-up and there were no indications of any severe liver disease in the histories. Consistent with the general view that cholestasis of pregnancy is a benign disorder (12) it is then unlikely that any significant number of the women have suffered permanent liver damage of clinical importance as a result of itching during pregnancy.

Cholestasis of pregnancy and cholestasis due to

oral contraceptives are considered to be related disorders and occur sometimes in one and the same woman (1). However, none of the women in the present series reacted with itching or jaundice to oral contraceptives. This is in accordance with Ramevik et al. (23) who demonstrated that oral contraceptives only rarely cause significant disturbances of liver function in women who have suffered itching and/or jaundice in pregnancy.

In a previous study (10) a familial occurrence of itching or jaundice in pregnancy was found in 7 of 16 women (44%) with typical recurrent cholestasis as against one of 25 women with non-recurrent jaundice. Among the present women 6 of 34 (18%) with recurrent itching knew of a relative with itching during pregnancy. The mildness of the syndrome in the present women and their relatives may help to explain the lower figure. It seems reasonable that a less dramatic clinical picture would be less known outside the immediate family circle.

It would then seem from the present follow-up study and from the follow-up study of jaundice in pregnancy (8-9-10-11) that women with recurrent cholestasis of pregnancy with itching as the most prominent symptom, and women with recurrent cholestasis with jaundice suffer from the same disorder and that the implications for the mothers and children are the same only differing in degree. Thus the hereditary predisposition and the tendency to early delivery were less pronounced in the former women than in the women with both itching and jaundice. However, both the prevalence of gallbladder disease in the women at follow-up and the incidence of asphyxia

at birth in their children were the same for all investigated women with recurrent cholestasis of pregnancy whether the symptoms had been itching and jaundice or itching alone

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LONG-TERM CONSERVATIVE THERAPY OF CHRONIC AV BLOCK

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Abstract Thirty-six of 98 patients with chronic advanced AV block have been treated conservatively by us, during the 5-year period 1966-70. Follow-up studies were made until Aug. 1973. When oral test doses of isoprenaline increased the heart rate without side effects, long-term isoprenaline abolished syncope in most patients both with normal and with widened QRS complexes. Eighteen patients died during the observation period, but death was in most cases unrelated to block. The study shows that conservative therapy may be used with fair success in selected patients.

Even if the indications for pacemaker therapy in chronic advanced atrioventricular (AV) block have been widened, many patients are still treated medically (1). The results of permanent pacing have been extensively published, but reports on long-term drug therapy are scarce. The purpose of the present report is to show the results of 5-year conservative therapy with follow-up in a regional hospital.

PATIENTS

In the 5-year period 1966-70, 98 patients with chronic symptomatic 2nd or 3rd degree heart block were admitted to our Department of Medicine. Follow-up examinations were made until Aug. 1973 for an average of 5 years, at least 2.5 years.

Thirty-six patients, 22 men and 14 women, and 56-92 years (mean 76, 11 patients <70, 11 70-80, 14 >80), were treated conservatively while 62 received permanent pacemakers. The reason for conservative treatment was mainly increased heart rate and clinical improvement after trial of oral isoprenaline therapy.

Thirteen of the 36 patients had no relevant previous or associated diseases. Myocardial infarction had occurred previously in 5 patients, but in at least one of these AV block had been recorded earlier. In the 5-year period advanced AV block developed during acute infarction in 105 patients, but persisted at 3 only (6).

Four patients had angina pectoris. None of these had syncope in association with chest pain. Ten patients had aortic valve disease, which in all was judged to be only slight or moderate. Of the remaining 4 patients, 2 had rheumatoid arthritis, 1 a history of rheumatic fever and 1 endomyocardial fibrosis.

One or more episodes of syncope had occurred in 22 of the patients and congestive heart failure was present in 18. Ten patients had only insignificant symptoms: slight dizziness, tiredness, etc.

Third degree AV block was present in 28 patients (QRS above 0.12 sec in 17 and below in 11) and 2nd degree AV block in 8 (QRS above 0.12 in 2 and below in 6). The AV block was intermittent in 16 patients (QRS above 0.12 in 6 and below in 10) and persistent in 20.

ECG patterns are shown in Table 1. Syncope had occurred in more than half of the patients both with normal and widened QRS. Syncope occurred equally frequently whether the block was intermittent or not.

The duration of AV block was unknown in 16 patients, 9 of whom were admitted to hospital after their first syncope. Among the 20 patients with known duration of heart block, 4 had a short history of a few days up to 3 weeks. In the remaining 16 patients AV block had lasted for 6 months up to 16 years (mean 7.4 years).

TREATMENT

Short-acting isoprenaline was given sublingually to 10 patients 4-6 times or more a day during the observation period. The average daily dose was 60 mg (37.5-90). Long-acting isoprenaline was given to 16 patients twice a day. The total daily dose varied from 60 to 40 mg. The response was first tested by counting the heart rate every 15 min or by continuous monitoring for 2 hours after oral short-acting isoprenaline first 7.5 mg and then, if necessary, after increasing doses up to 30 mg. The i.v. isoprenaline test (2) was not used routinely as occasionally good i. heart rate response was not followed by response after sublingual doses. Ten patients received diuretics and 8 digitalis in addition to isoprenaline while 10 had such specific symptoms that no therapy was given.

have been reported in up to nearly 25% (7). We have observed breakage of the electrode in about 10% of the paced patients often after several years. Development of unexplained congestive heart failure after pacemaker implantation has been reported (3).

Isoprenaline may also cause discomfort and complications such as tachycardia, sweating and tremor which have been noted in 20–55% of cases (1). In our long-term treated patients unpleasant side-effects were not observed probably due to the rather low doses compared with many other studies.

How frequently is isoprenaline therapy sufficient? Among all of our 98 patients with advanced chronic AV block isoprenaline was attempted in 41. Fifteen of them had to be treated with pacemaker either because of no effect or because of side-effects especially ventricular or supraventricular arrhythmias.

In conclusion pacemaker therapy may offer the best results as regards long-term prognosis in chronic symptomatic advanced AV block. But it may not be feasible to treat all patients with pacemaker. The present study shows that long-term therapy with isoprenaline can be used with fair success and few side-effects if the heart rate increases and the clinical condition improves after relatively low oral doses.

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REMOVAL OF EXOGENOUS PLASMA TRIGLYCERIDES IN SPLANCHNIC VISCERA IN MAN DURING ANESTHESIA

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Abstract The role of the splanchnic viscera and the liver in removal of an artificial fat emulsion Intralipid® has been studied in anesthetized man. Ten males and six females were studied. During operation for cholecystectomy catheters were placed into an artery the portal and the hepatic veins. During a constant i. v. infusion of Intralipid® the concentration of the fat emulsion was determined by nephelometry in blood plasma sampled at intervals from these three vessels. Splanchnic plasma flow was estimated simultaneously. During the infusion constant concentrations of Intralipid® was found in the blood. The splanchnic flow was not affected by the infusion of the fat emulsion. The difference in mean arterio-portal concentration of Intralipid® was $69 \pm 15 \mu\text{mol/l}$ at 20 and 31 min, corresponding to a removal of $5.3 \pm 1.2\%$ of the arterial concentration ($p < 0.001$). The amount of infused Intralipid® removed per minute was $35 \pm 8 \mu\text{mol}$, corresponding to $25 \pm 6\%$ of the infused quantity ($p < 0.001$). No significant changes of tissue concentrations were observed between the portal and the hepatic veins.

In studies of the role of the splanchnic region in the turnover of triglycerides (TG) from the blood stream in man (4-26) the technique of hepatic vein catheterization has been used. With this method the TG uptake and release both by the liver and the splanchnic viscera are estimated. However the idea has been put forward that part of the TG eliminated from the blood may be taken up by the splanchnic viscera (4). There are several reasons for this possibility. It is well known that the omental adipose tissue is metabolically very active and several analyses of the lipolytic activity have demonstrated higher activity in omental adipose tissue than in subcutaneous fat (9, 22, 23). Chylomicrons are believed to be removed both by liver and adipose tissue as intact particles (24-27, 28) or after hydrolysis (15, 19). Recent investiga-

tions have suggested that the liver is a minor site of direct incorporation of chylomicron (1, 14).

Olivecrona and Belfrage (77) have demonstrated that in the carbohydrate-fed rat intact chylomicrons can be removed also in the small intestine. In order to further evaluate the role of the splanchnic region in man with exclusion of the liver it is necessary to analyse portal blood.

The L.V. fat tolerance test (IVFTT) has recently been introduced as a simple test which determines the elimination rate from the blood stream of exogenous TG using the artificial fat emulsion Intralipid® (Vitrum Stockholm) (3, 11). This test also significantly reflects endogenous plasma TG turnover (29). Little information has been available about the fate of the emulsion in man during its elimination. In a previous study we have demonstrated that 6% of the arterial concentration of Intralipid® was taken up by the human heart (10). The aim of the present study was to study whether Intralipid® which in several respects resembles chylomicrons (3, 8, 17) was taken up in the splanchnic region and in the liver.

SUBJECTS DESIGN OF STUDY AND METHODS

Subjects

Two studies were performed: a pilot study of 5 male patients aged 37-58 (study I) and a second of 6 females aged 25-57 and 5 males aged 37-58 (study II).

The patients were admitted to the Department of Surgery for cholecystectomy because of gallstones and were studied after their informed consent. All patients were free from symptoms and the surgery was elective. Furthermore none had symptoms suggesting metabolic disorder or were on medication expected to affect lipid metabolism. Fasting plasma TG and cholesterol were determined before hospitalization. Three males in study I and four males and one female in study II had

Table 1 Clinical data of the patients in study II (mean \pm S.E.M. ranges within parentheses)

Pat. no	Sex	Age (yr)	B wt (kg)	Weight/height index (kg/cm ²)	Peroperative plasma lipids		Plasma volume (l)
					TG (mmol/l)	Cholesterol (mg/100 ml)	
1	♀	34	80	1.13	0.92	186	2.80
2	♀	35	80	1.27	1.25	240	2.80
3	♂	38	80	0.96	2.07	13	2.95
4	♀	46	63	0.93	1.17	59	2.60
5	♀	45	50	0.94	1.97	58	2.35
6	♀	57	70	1.17	42	263	2.50
7	♂	51	102	1.31	3.06	216	3.70
8	♂	47	81	1.16	3.16	266	2.95
9	♂	53	86	1.12	2.69	214	3.10
10	♀	25	54	0.79	0.72	211	2.30
11	♂	58	70	0.97	1.79	226	2.85
Females		40 \pm 5 (25-57)	66 \pm 5 (50-80)	1.04 \pm 0.07 (0.79-1.37)	1.40 \pm 0.26 (0.72-2.42)	236 \pm 13 (186-263)	2.56 \pm 0.09 (2.35-2.80)
Males		49 \pm 3 (38-58)	84 \pm 5 (70-102)	1.10 \pm 0.07 (0.96-1.31)	2.55 \pm 0.27 (1.79-3.16)	227 \pm 10 (213-266)	3.13 \pm 0.15 (2.95-3.70)

fasting plasma TG concentration above 2 mmol/l. The clinical data of the subjects in study II are summarized in Table 1.

Design of study

IVFTT was performed in all patients 1-4 days before operation. Patients in study II were given an injection of 5-8 μ C ¹²⁵I-albumin concomitantly with the IVFTT to permit determination of the plasma volume and detection of possible shifts in plasma water concentration between arterial and venous blood.

Study I was aimed only at detecting differences in arterio-portal concentration of exogenous plasma TG using constant infusion of Intralipid®. Arterial and portal blood was drawn simultaneously 5 times (2, 3, 10, 20 and 30 min after the start of the infusion) and the concentration of the fat emulsion was determined 10 times on each sample.

Study I utilized study II designed to quantify the splanchnic uptake of exogenous TG. For this purpose the hepatic vein was catheterized and blood was sampled for estimation of hepatic blood flow, concentration differences across the liver. The design and consecutive order of the different procedures in study II are given in Fig. 1.

Anesthesia
All patients were studied in the morning after overnight fast and premedication with atropine and pethidine (ACO, Stockholm, Sweden). The patients were anaes-

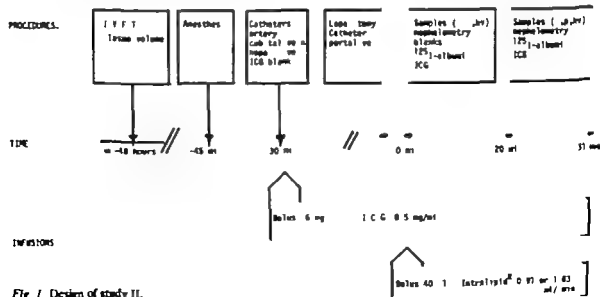


Fig. 1 Design of study II.

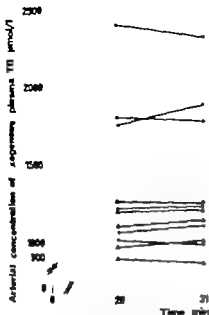


Fig. 2 Arterial concentrations of exogenous plasma TG in study II at 20 and 31 min. \circ = Intralipid[®] infusion rate of 1.63 ml/min, Δ = of 0.91 ml/min.

thetized with droperidol plus fentanyl (Dripdol[®] and Leptranal[®] Leo Helsingborg, Sweden). They were intubated and ventilated by the Engström respirator (LKB Stockholm) with a mixture of 30% oxygen and 70% nitrous oxide during the study. When the patients had been anesthetized the studies were performed and after that the surgical procedure was completed. Only saline was given during the study as a slow drip infusion. All patients were discharged from the hospital without complications or blood transfusions.

Calculations

In all patients an arterial catheter was placed in the right brachial artery. In study II right hepatic vein was catheterized under fluoroscopic guidance from the right arm (4). Ten per cent Intralipid[®] and indocyanine green (ICG) (Cardio-Oreen[®] Hynson Westcott & Dunning, Baltimore) were infused into peripheral veins by means of constant rate infusion pumps. The infusion rates of Intralipid[®] were either 1.63 or 0.91 ml/min after a bolus dose of 40 ml. The infusion rate was chosen on the basis of the fractional elimination rate k_e calculated from the IVFTT performed before the operation. Patients with k_e value below 4%/min received the slow infusion rate.

The portal vein was catheterized as described by Gonzalez Carballales (16). A right-sided subcostal incision was made for the cholecystectomy. Ligamentum teres hepatis was easily identified and the rudiment of the umbilical vein was exposed. This occluded vein was incised and easily recanalized with blunt probes of 2–3 mm diameter. When the junction between the

umbilical vein and the portal vein had been opened polyethylene catheter (PE 260) was inserted. The tip of the catheter was positioned in the left branch of the portal vein close to a umbilical junction to permit easy blood sampling. No heparin was given during the study. All catheters were kept patent either by slow saline drip or by intermittent flushing with saline.

Blood sampling

Initially 3 ml arterial blood was drawn for determination of fasting plasma TG and cholesterol. Furthermore blood was collected from the artery the portal vein and the hepatic vein (only study II) for estimation of blank values for the subsequent ICG and Intralipid[®] determinations. After the start of the Intralipid[®] infusion blood was sampled at intervals. 15 ml blood was sampled simultaneously from the artery the portal and hepatic veins (only study II) for nephelometry ICG and 125 I-albumin determinations. Only dry plastic syringes were used without additives. Samples were drawn at 1, 5, 10, 24, 27 and 31 min in study I and at 10 and 31 min in study II. The portal catheter was not flushed until 11 samples from the hepatic vein had been drawn. Altogether less than 50 ml blood was taken from each patient.

Intravenous fat tolerance test

IVFTT has previously been described in detail (14). One ml/kg b.wt. of 10% Intralipid[®] was given i.v. as pulse injection and blood samples were taken every 5 min during 40 min. The light scattering index (LSI) of the plasma samples diluted 1:100 was read in a Thorp microspectrophotometer and plotted in semi-logarithmic system, where a straight line was found. The slope of the line was calculated and called k_e (%/min). A standard curve was prepared from each bottle of Intralipid[®] for calculation of the TG concentration from the LSI obtained by nephelometry.

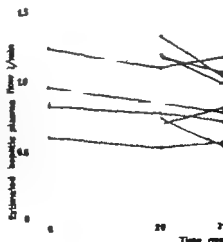


Fig. 3 Estimated hepatic plasma flow during constant infusion of Intralipid[®].

Nephelometry

From each arterial portal and hepatic vein blood sample, 10 times 40 μ l plasma was diluted 1:100 in saline for replicate nephelometry determinations (30). All samples (up to 120 tubes) were then randomized and the technician reading the nephelometer LSI did not know the origin of the samples. The average reading for the fasting plasma sample from the artery the portal and the hepatic veins was subtracted as a blank from the respective sample reading. Means \pm S.E.M. of the LSI were calculated and LSI was converted to μ mol/l Intralipid[®] TG by means of the standard curve and an assumed mean molecular weight for Intralipid[®] TG of 878.

Chemical determination

Serum TG and cholesterol before operation were determined by an AutoAnalyzer technique (2, 20) in the presence of nos. 1 and 2) the plasma TG concentration was determined in ten Folch chloroform-methanol extracts of arterial and portal blood samples respectively (7). The chloroform phase was washed and by subsequent AutoAnalyzer technique the TG concentration was determined on triplicates of each such extract (7-10).

As the Intralipid[®] content of the samples would have interfered with the spectrophotometric determination of ICG the samples were centrifuged in the ultracentrifuge at 36 000 rpm for 40 min. The ICG was then determined on plasma in a Beckman spectrophotometer as suggested by Nielsen (23). The plasma volume was determined with ¹²⁵I-albumin (3.1) F ribersmore the ¹²⁵I-albumin concentration was determined in the plasma replicates from each sample in study II.

Calculation

The calculation of the fractional removal rate k in the IVFTT has previously been described (11). The calculation of the fractional removal rate of Intralipid[®]

during the constant infusion was performed according to the formula

$$k = \frac{\text{Infused Intralipid}^{\circ} \mu\text{mol/min}}{A_{\text{LM}} \times C \times PV}$$

where A_{LM} is the mean arterial plasma LSI minus blank value C is a factor for the conversion of LSI to Intralipid TG (μ mol/l) and PV is the plasma volume (l).

The blood volume on the day of operation was assumed to be the same as during the IVFTT. A steady state concentration of the fat emulsion in blood was found within 20 min of constant infusion (Fig. 2). The splanchnic blood flow was estimated by measuring ICG concentration in the artery and the hepatic vein as described previously (6). Corrections due to a small but significant arterio-portal difference in ICG concentration will be presented and discussed below. Of the hepatic blood flow 80% was assumed to come from the portal vein and 20% from the hepatic artery.

The elimination (E) of Intralipid[®] in the splanchnic region except the liver was calculated according to the formula

$$E = (A_{\text{LM}} - P_{\text{LM}}) \times C \times \text{ESPF} \times 0.8 \mu\text{mol/min}$$

where P_{LM} is the mean portal plasma LSI minus blank loss and ESPF is the estimated splanchnic plasma flow (l/min).

RESULTS

The constant infusion of Intralipid[®] resulted in a constant concentration of fat particles in the blood stream as determined by nephelometry (Fig. 2). The fractional removal rate determined from the

Table II Arterial (a) concentration of exogenous plasma TG differences in arterio-portal (a-p) concentration and splanchnic removal of the infused Intralipid[®] (nephelometric determinations in μ mol \pm S.E.M.)

Pat. No.	20 min			31 min			70 min		
	a (μ mol/l)	a-p (μ mol/l)	% of a	a (μ mol/l)	a-p (μ mol/l)	% of a	Removal (μ mol/min)	% of infused Intralipid [®]	
1	1 010 \pm 10	70 \pm 10***	7.9	980 \pm 10	60 \pm 10***	5.8	41	22	
2	1 040 \pm 10	70 \pm 10*	6.7	1 110 \pm 10	40 \pm 10*	3.6	-	-	
3	970 \pm 20	10 \pm 20**	1.1	1 020 \pm 10	80 \pm 10*	8.3	7**	7	
4	1 810 \pm 10	150 \pm 20*	8.3	1 790 \pm 10	100 \pm 10***	5.7	60***	32	
5	1 760 \pm 10	-10 \pm 20**	-0.5	1 890 \pm 10	40 \pm 10*	2.1	-8**	-4	
6	1 200 \pm 10	-30 \pm 20**	-3	1 220 \pm 10	10 \pm 10**	0.7	-23**	-23	
7	870 \pm 10	10 \pm 10**	1.7	-	-	-	13**	1	
8	1 100 \pm 10	80 \pm 10***	7.6	1 140 \pm 10	90 \pm 10***	7.7	56	54	
9	1 270 \pm 10	70 \pm 20*	7.7	1 40 \pm 10	40 \pm 10*	1.6	54	52	
10	2 410 \pm 10	-10 \pm 20**	-0.7	320 \pm 10	130 \pm 30***	1.8	-16**	-9	
11	1 220 \pm 10	100 \pm 20***	16.7	1 240 \pm 10	70 \pm 20**	20.5	118***	64	
Mean \pm S.E.M.	1 330 \pm 140	55 \pm 21	4.9 \pm 1.7*	1 390 \pm 140	88 \pm 22***	5.8 \pm 1.8	30 \pm 14**	20 \pm 9*	
Mean	(20+31 min = 1)						33 \pm 8*	(μ mol/min)	

* $p > 0.05$ $p < 0.05$ $p < 0.01$ ** $p < 0.001$ based on t -test of difference between two means.

preoperative IVFTT (k_2) was compared to the k_2 value during the infusion in study II. The mean k_2 was 4.73 ± 0.69 %/min and the mean k_2 6.03 ± 0.45 %/min ($p > 0.05$). The constant infusion of ICG in the patients of study II resulted in a constant concentration in arterial as well as hepatic vein blood. The calculated hepatic plasma flow was not significantly changed during the experimental period in this study (Fig. 3).

The pilot study

The LSI of portal blood plasma was significantly lower than the corresponding arterial values in 1 of the 5 calculated differences in the 5 patients in two arterio-portal pairs a significant negative difference of concentration was found (5.3 and 6.8 % of the arterial concentration). The difference in mean arterio-portal concentration (\pm S.E.M.) was 30 ± 9 μ mol/l ($p < 0.01$) corresponding to a removal of 1.51 ± 0.67 % ($p < 0.05$) of the arterial Intralipid[®] concentration.

Study II

From the 11 patients in this group 1 pairs of arterio-portal samples were obtained at 20 and 31 min. In 16/21 pairs a significant positive difference in arterio-portal concentration of Intralipid[®] as determined by nephelometry was found. No significant negative differences in arterio-portal concentration were observed. No differences in

mean arterio-portal or portal-hepatic venous concentration of 51 I-albumin were found. The mean arterial ICG concentration was 0.97 mg/l and the mean portal ICG concentration 0.89 mg/l ($p < 0.01$).

The difference in mean arterio-portal concentration of Intralipid[®] TG was 55 ± 21 μ mol/l at 20 min ($p < 0.05$) and 84 ± 22 μ mol/l ($p < 0.01$) at 31 min (mean \pm S.E.M.). The individual values for each subject are given in Table II. These differences in arterio-portal concentration corresponded to a reduction of 4.9 ± 1.7 % ($p < 0.05$) of the arterial concentration at 20 min and 5.8 ± 1.8 % ($p < 0.05$) at 31 min. In patients 1 and 2 the differences in arterio-portal TG concentration as determined by nephelometry were 70 ± 20 , 40 ± 20 , 70 ± 10 and 60 ± 10 μ mol/l. The corresponding figures obtained by the chemical method were 9 ± 7 , 46 ± 7 , 70 ± 13 and 33 ± 10 μ mol/l respectively.

The difference in mean concentration of exogenous TG between the portal and hepatic veins, -18 ± 30 μ mol/l at 20 min and -17 ± 19 μ mol/l at 31 min are not statistically significant. The amount of infused Intralipid[®] removed in the splanchnic region during steady state conditions was calculated. At 20 min 30 ± 14 μ mol/min (S.E.M.) was removed corresponding to 20 ± 9 % ($p < 0.05$) of the infused amount of Intralipid[®]. The corresponding figure at 31 min was 41 ± 9 μ mol/min 29 ± 6 % of the infused Intralipid[®] ($p < 0.01$). There was no correlation between the two infusion rates and the absolute or percental removal of Intralipid[®] or between infusion rates and the amount removed per minute. The fractional removal rates as determined by k_2 and k_2 the serum TG and cholesterol concentrations and the weight/height index did not correlate significantly to the differences in absolute or percental arterio-portal concentration or the uptake per minute of exogenous plasma TG.

DISCUSSION

Methodology

In this study the changes in concentration of Intralipid[®] were determined by nephelometry. As nephelometry is a method by which the light scattering of plasma is studied, it is essential to verify that changes in light scattering agree with changes in concentration of exogenous plasma TG. Nephelometry is especially sensitive in the recognition

31 min	
Removal (μ mol/min)	% of infused Intralipid [®]
31 ⁺⁺	17
42 ⁺⁺	41
39 ⁺⁺	21
26 ⁺⁺	14
9 ⁺⁺	9
51 ⁺⁺⁺	52
32 ⁺⁺⁺	31
32 ⁺⁺	17
161 ⁺⁺⁺	56
41 \pm 9 ⁺⁺	29 \pm 6
25 \pm 6	(% of infused Intralipid [®])

of larger particles since an increase of the particle radius by a factor is equivalent to a LSI increase by at least the square of the factor (30). A reduction of Intralipid® particle size during the passage through the splanchnic region would thus present as a reduction of light scattering although the plasma TG concentration remained unchanged. However, in earlier studies it has been shown that there is an excellent agreement between nephelometry and chemical methods (21). In the present study a comparison was also made in two cases between nephelometry and chemical determination of plasma total TG after extraction with chloroform-methanol and washing of the chloroform phase. The comparison indicates that the changes in nephelometric concentration between the artery and the portal vein reflected a decreased concentration of Intralipid®. As discussed in detail in a previous study (10) the small differences in concentration observed make it mandatory to apply methods with high precision. If a technique for separation of exogenous from endogenous TG involving too large a methodological error had been applied in the present study it is possible that statistically significant differences in concentration would not have been observed.

The current finding of a significant arterio-portal difference of 0.03 mg ICG/l, i.e. on an average 3% of the arterial concentration, deviates from the observations of Viallet et al. (31) who reported non-significant differences in arterio-portal concentration of both ICG and bromsulphthalein. The extrahepatic elimination of ICG has been considered insignificant for practical purposes (12) but if differences in ICG concentration were to indicate a water shift, the differences in TG concentration would be largely influenced in the present study. As no difference in arterio-portal concentration of ¹²⁵I-albumin was found this argues against such a mechanism and makes an extrahepatic ICG removal in gastrointestinal or gas probable.

Splanchnic removal of exogenous plasma TG

Under the assumption that the nephelometric determinations of differences in Intralipid® concentration reflect a removal of the fat emulsion the following reflections may be made. During the passage from the arterial system in the portal vein a reduction of the exogenous plasma TG concentration by means of 5.3% of the arterial

concentration was observed. In this study however no significant difference in concentration of exogenous plasma TG was found across the liver the grand mean being $-3 \pm 3\%$. The passage through the splanchnic region to the portal vein involves a number of tissues such as the gastrointestinal tract, the spleen and the omental adipose tissue. The design of the study does not make it possible to conclude which tissues preferentially removed Intralipid® from the circulation. However it has been indicated that the reticuloendothelial system does not take up Intralipid® to any great extent (13). This is supported by our finding that there was no significant uptake between the portal and hepatic veins.

The liver is known to contain a substantial number of phagocytic Kupffer cells which therefore seem to be without major importance for the removal of Intralipid® under the conditions of our study. Therefore it is possible that the spleen as well may be of minor importance. Although several studies have demonstrated a chylomicron TG removal in the liver others have stressed the importance of adipose tissue rather than the liver in the metabolism of chylomicron TG (1, 77). Felt (14) has even suggested that the liver does not take part at all in the direct metabolism of chyle TG.

The amount of chylomicron TG removed in the liver by the carbohydrate-fed rat has been estimated to be about 1/3 of a lipid load, the rest being mainly removed in the adipose tissue (77). Our present finding demonstrates how important it is to study the role of the various parts of the splanchnic and hepatic systems separately and is in agreement with studies showing little hepatic uptake of chylomicron TG. Previous studies on the omental adipose tissue indicate that this tissue is very active in lipid metabolism and may possibly be responsible for the removal.

The extraction of the Intralipid® emulsion in the myocardium has previously been studied (10) and a decrease of the exogenous plasma TG concentration by grand means of 6% was reported. In the present study we have demonstrated that the Intralipid® emulsion may be taken up in the splanchnic region as well. The data on estimated splanchnic plasma flow make it possible to calculate roughly the fraction of the infused Intralipid® that is taken up in the splanchnic region. Even if the estimated hepatic plasma flow is cor-

rected to give 3% higher values since the portal ICG concentration was lower than the mean arterial concentration the figures for the uptake of Intralipid® in the splanchnic region are very little affected.

In this study it was of interest to note that the infusion of the fat emulsion Intralipid® did not result in flow changes in the splanchnic region. This is in agreement with earlier findings in dogs and in contrast to flow changes induced by other outflows, such as amino acids which increase the splanchnic plasma flow considerably (18).

CONCLUSION

By measurements with nephelometry of differences in arterio-portal concentration of i.v. Intralipid® the role of the splanchnic region in removal of this fat emulsion has been studied in anesthetized patients during operation for cholecystectomy. The present study suggests that exogenous plasma TG can be removed significantly on passage through the splanchnic viscera. No significant net removal of Intralipid® was observed across the liver. The grand mean \pm S.E.M. uptake across the splanchnic bed was $5.3 \pm 1.1\%$ of the arterial concentration. This corresponded to a removal of $3 \pm 6\%$ of the Intralipid® infused per minute.

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THE EFFECT OF A LONG-ACTING GLUCAGON PREPARATION ON ENDOCRINE AND METABOLIC RESPONSES IN ACUTE MYOCARDIAL INFARCTION

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Abstract In acute myocardial infarction (AMI) the availability and the insulin-dependent transportation of glucose can possibly influence the extent of myocardial damage and the incidence of arrhythmias. Glucose metabolism is affected by several endocrine changes in connection with an AMI. Some of these changes have been studied in 43 patients with an uncomplicated AMI. A slight hyperglycemia was seen, with an increased catecholamine secretion and growth hormone secretion but with no reduction in the basal insulin secretion. The treatment of 27 of the patients with a zinc-protein-glucagon (ZPG) suspension, s.c. 8 mg i.i.d. changed the natural response to an AMI only through a slight increase in the plasma growth hormone and insulin levels. The clinical course was not affected by ZPG. It was tolerated without side-effects. It is concluded that treatment with ZPG is not indicated in uncomplicated cases of AMI.

Acute myocardial infarction (AMI) is accompanied by several endocrine and metabolic changes usually in relation to the severity of the myocardial damage and the clinical course. These changes include an increased secretion of catecholamines (6, 15, 16, 18, 19, 25, 43), cortisol (6, 43), growth hormone (6, 25) and glucagon (24). Insulin secretion is depressed during the first days (6, 25, 31) and elevated during the 6th-14th days (25) after AMI. Glucose tolerance is impaired and hyperglycemia is a common finding (5, 4). Circulating free fatty acids (FFA) are increased (6, 16, 18, 21, 43, 44).

These changes can influence the extent and severity of myocardial necrosis (7, 31, 37) and alter myocardial contractility in an acutely failing heart (1, 51). In the ischemic myocardium glucose serves as primary substrate for energy production (7, 40), and the rate of its uptake is dependent on

the availability of insulin (34). Diminished insulin secretion together with increased catecholamine and probably also with increased growth hormone secretion, account for elevated FFA levels (1, 40) which have often been observed to be associated with a high incidence of arrhythmias (16, 17) even if some reports have not confirmed this association (38, 40, 44).

The treatment of AMI with glucose, insulin and potassium (GIK) was suggested by Sodi-Pallares et al (46, 48). Although clinical experiments with this regimen or its modifications have shown a reduction in acute mortality only in a few minor studies (11, 33, 41) and no effect in many larger series (4, 17, 36, 49) the importance of further studies for the reevaluation of this kind of treatment has also recently been emphasized (1, 8, 11, 17, 28, 3, 47, 49).

Glucagon activates glucose production from glycogen in the liver (10, 52) and it may be responsible for the hyperglycemia following AMI (24). It is also a potent stimulator to insulin release (3, 10, 50). Glucagon is used in the treatment of cardiogenic shock and intractable heart failure (13, 37) because of its positive cardiac inotropic effect, and also because of the possible beneficial metabolic actions, as the GIK regimen is also used experimentally (2, 28, 51). Even if large doses of glucagon increase myocardial oxygen consumption and may enlarge the size of the infarct (31) there is a sufficient increase in myocardial blood flow to meet the growing myocardial demands (14, 30, 29, 37). The inotropic effect is usually attained without any increase in the incidence of arrhythmias (13, 39) and with facilitated atrio-ventricular conduction (39).

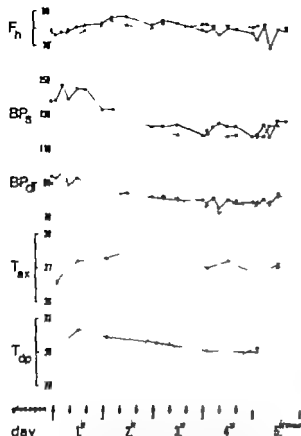


Fig. 1 Mean heart rate (F_h), systolic (BP_s) and diastolic (BP_d), and axillary (T_{ax}) and peripheral skin temperature (T_{skin}) of 22 glucagon-treated (—) and 11 control patient (---). The arrows show the times of injection.

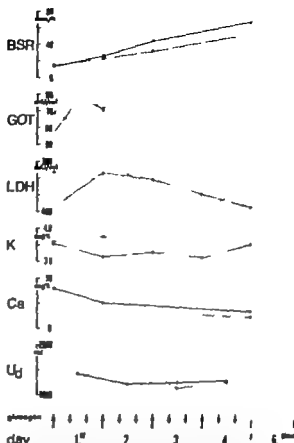


Fig. 2 Mean blood sedimentation reaction (BSR), serum GOT and LDH enzymes, plasma potassium (K), serum calcium (Ca) and daily urine volume (U) of 22 glucagon-treated (—) and 11 control patients (---).

This study was undertaken in order to investigate the effect of glucagon administration on glucose production and insulin release in patients with uncomplicated AMI. Concomitant excretion of catecholamines and growth hormone secretion was also studied in AMI patients with and without glucagon treatment. The preparation used was a zinc-protamine-glucagon suspension (ZPG) with prolonged action (3–50) given subcutaneously 8 mg t.i.d.

MATERIAL AND METHODS

Patients and general measures. All of the 33 male patients had definite AMI. Diabetic patients on insulin patients with serum creatinine values above 1.5 mg/100 ml and patients with uncontrollable arrhythmias or severe left heart failure on admission were not included. The patient were treated in the CCU of Turku University Hospital, which they had entered not earlier than 4 hours before the experiment started at 7 a.m.

The median time which had elapsed since the onset of typical chest pain was 17 hours for the 11 patients in the control group and 23 hours for the 22 patients in the glucagon group. The means \pm S.D. of age and weight

these groups were 51.9 ± 9.0 and 52.2 ± 7.0 years and 68.5 ± 8.3 and 74.7 ± 11.2 kg respectively. Two smaller groups of 5 patients each, one control group and another treated with glucagon injections at 8-hour intervals, were followed closely with blood samplings every 2 hours for 24 hours. With the others the experiment was continued for 4 days with glucagon injections at 8 a.m., 4 p.m. and midnight. The sampling procedure is presented in Figs. 1–3. No placebo injections were used. An i.v. drip of 0.9% NaCl was set instead of glucose infusion for the first 1–2 days. The average amount of infusions was 780 ml during the 1st day and all liquids given totalled 1510 ml . The use of diuretics was restricted to the absolute minimum. The patients were allowed to take light meals at 11 a.m. and 4 p.m. Unrestricted sitting was on an average begun on the 3rd day and standing was allowed on the 5th day.

Glucagon. Subcutaneous injections of 8 mg ZPG were administered. This preparation of slow-acting

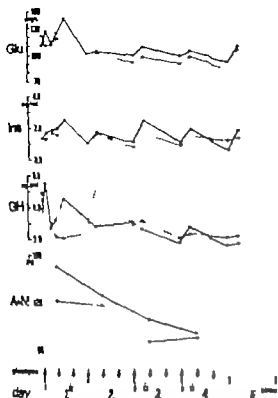


Fig. 3 Mean blood glucose (Glu), plasma immunoreactive insulin (Ins), growth hormone (GH) and daily urinary excretion of adrenaline and noradrenaline (A+N) of 22 glucagon-treated (—) and 11 control patients (---).

glucagon (3.90) was manufactured and supplied by the Novo Industri A/S Copenhagen.

Clinical measurements. Heart rate (cardiotachometer), BP (cuff method), axillary and skin (dorsal toe) temperature were measured by standard methods of the CCU. Normal reference values were for GOT <19 mU/L, LDH <240 mU/ml, plasma potassium 3.3–4.8 mg/100 ml, serum calcium 8.8–10.8 mg/100 ml, blood glucose (fasting) 60–90 mg/100 ml and 24-hour urine adrenaline+noradrenaline <100 µg. Urine catecholamines are determined fluorimetrically by Pk-Larsson method as described by Manninen (30).

Plasma insulin and growth hormone. Blood samples, drawn into heparinized tubes, were centrifuged and plasma was stored at -20°C until analysis. Plasma insulin and growth hormone were determined by the immunoradiometric radioimmunoassay method (34). The standard preparation for insulin was human insulin WHO 166/04 and for growth hormone NIH-GH-HSL 394.

RESULTS

Clinical events. No deaths occurred either in the control group of 11 patients or in the glucagon group of 22 patients. No cases of severe cardio-

genic shock, severe heart failure, heart arrest or ventricular tachycardias were encountered. The numbers of patients with special therapeutic measures in the control and glucagon groups were as follows: lidocaine infusions 3 and 7, digitalis 4 and 6, endocardial pacing 0 and 1 patients respectively. No differences were seen in the number of injections of pethidine or pentazocine, or in attacks of vomiting.

Clinical measurements. The mean values of clinical measurements and of serum determinations are presented in Figs. 1–4. BPs, particularly systolic and pulse pressure, were non-significantly higher during the first day on glucagon. The peripheral skin temperature was higher ($p < 0.05$) in the glucagon than in the control group. The lower plasma potassium and the higher serum calcium in the glucagon group than in the control group were not significant findings.

Endocrine changes (Fig. 3). A slight hyperglycemia as a response to the AMI on the morning of the first day of the experiment was found prior to the treatment in 8 of the 11 controls and in 16 of the 22 patients treated with glucagon. The mean blood glucose level of the patients treated with glucagon was slightly but not significantly higher than that of the controls. A fluctuation of blood glucose level was found in connection with the meals.

Plasma insulin levels followed the changes in blood glucose. There was no difference any time in the study between the two groups and the fasting plasma insulin values were within normal limits. In the out-patient groups studied at 2-hour intervals during the first day (Fig. 4) the plasma insulin level was significantly higher at 10 a.m. and 2 p.m. in the glucagon group than in the control group.

Plasma growth hormone levels varied greatly in individual patients with high secretion peaks as seen in Fig. 4. In the minor groups a significant increase in growth hormone levels was found 4 hours after the first glucagon injection. In only 3 of the 10 patients was a sleeping peak found. The growth hormone levels were quite similar in the major glucagon and control groups on the 3rd day of the study and afterwards.

The total daily urinary adrenaline and noradrenaline excretion was not significantly higher in the groups treated with glucagon than in the control groups. As expected a continuous and

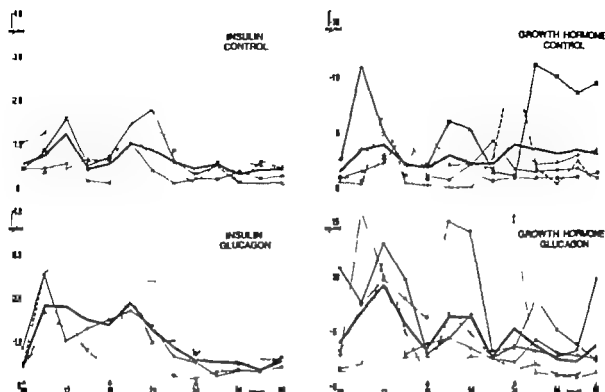


Fig. 4 Individual levels of plasma immunoreactive insulin and growth hormone in 5 glucagon-treated and 5 control patients with AMI during the first 24 hours of

the experiment. Mean levels are shown by the thick lines, the times for glucagon injections by the arrows.

significant decrease of the 4-hour catecholamine excretion was found in both major groups.

The results were also examined in the light of the individual levels found. Thus the 12 patients treated with glucagon were divided into two subgroups of 11 patients each according to higher or lower values during the first day of the study for urinary excretion of catecholamines, mean levels of blood glucose, plasma insulin or growth hormone and serum GOT. The comparison of the endocrine findings in these two subgroups is shown in Table 1. The patients with a higher blood glucose tended to have a higher catecholamine excretion and a higher plasma insulin level but not a higher growth hormone level. When the material was divided according to the insulin or growth hormone levels, no differences in the other hormonal parameters were found.

DISCUSSION

The metabolic and endocrine effects of glucagon were studied in patients with an uncomplicated AMI.

The changes we found in connection with the

AMI itself were similar to those previously reported (see Introduction), e.g. hyperglycemia, increased levels of growth hormone and an increased excretion of catecholamines. These changes were most pronounced during the first 4-hour period of the study. As the median time from the onset of pain to the start of the experiment was 17 hours for the controls and 23 hours for those under glucagon treatment, the peak of rapidly ensuring reactions had in some cases possibly been passed. The catecholamine secretion increases already during the first few hours following an AMI (15, 43), as does hyperglycemia due to catecholamines and cortisol secretion (25). No depression in basic insulin secretion was found in our study. This was not surprising, since a marked depression has previously been found in AMI, complicated by heart failure or cardiogenic shock (51) or in chronic circulatory failure (12, 45).

When the endocrine response to the AMI was examined by checking up the plasma levels at 2-hour intervals during the first 4 hours in 10 patients, the insulin secretion was found to be increased in a normal way after relatively light

Table 1. Endocrine findings in the high and low value halfgroups of glucagon-treated patients (means \pm S.E.M.)

GH = growth hormone, A+N = adrenaline and noradrenaline

Basal for division	GOT (mU/ml)	Glucose (mg/100 ml)	Insulin (ng/ml)	GH (g/ml)	A+N (μ g)
GOT ≥ 70.0					
High	101.2 \pm 7.41	7.27 \pm 0.30	1.78 \pm 0.33	3.14 \pm 0.40	168.6 \pm 48.5
Low	36.6 \pm 8.11	6.27 \pm 0.30	1.88 \pm 0.36	3.96 \pm 1.21	184.5 \pm 43.4
Glucose ≥ 6.60					
High	7.27 \pm 0.30	8.13 \pm 0.77	2.12 \pm 0.47	3.10 \pm 0.77	208.0 \pm 45.0
Low	6.27 \pm 0.30	5.92 \pm 0.15	1.38 \pm 0.24	3.73 \pm 0.94	123.8 \pm 26.3
Insulin ≥ 1.75					
High	1.78 \pm 0.33	6.57 \pm 0.37	2.51 \pm 0.35	3.39 \pm 0.99	198.3 \pm 58.9
Low	1.88 \pm 0.36	7.59 \pm 1.00	1.01 \pm 0.17	3.88 \pm 0.95	193.0 \pm 33.3
GH ≥ 3.5					
High	3.14 \pm 0.40	6.84 \pm 0.36	1.66 \pm 0.27	4.19 \pm 0.94	173.0 \pm 34.7
Low	3.56 \pm 1.21	7.21 \pm 0.91	1.80 \pm 0.49	1.72 \pm 0.19	179.4 \pm 48.2
A+N ≥ 120.0					
High	168.6 \pm 48.5	7.71 \pm 0.79	1.66 \pm 0.39	3.65 \pm 0.94	261.4 \pm 34.6
Low	184.5 \pm 43.4	6.34 \pm 0.25	1.84 \pm 0.23	3.20 \pm 0.79	77.3 \pm 8.3

onists. A similar observation was made concerning the secretion of growth hormone reflecting a paradoxical elevation in the postabsorptive state (9) and possibly also the additional exertion of eating in bed. A strong sleeping peak in growth hormone was seen during the hours before midnight in only 3 of 10 patients. A lack of or disturbed sleep in the CCU was found in connection with the absence of sleeping peak in most of the others.

Plasma levels of endogenous glucagon were not determined in this study. Secretion was probably increased in patients with elevated blood glucose levels as Lamiado et al. (24) found in their series of AMI patients. In our series the fasting blood glucose was over 90 mg/100 ml in 8 of 11 patients in the control group and in 16 of 22 patients in the group treated subsequently with glucagon. The "stress" hormones, catecholamines, cortisol and growth hormone also promote hyperglycemia by gluconeogenesis and glycogenolysis and the relative contribution of these hormones is largely unknown. Endogenous secretion of glucagon is, however, diminutive in comparison with the concentrations produced by the usual therapeutic administration of glucagon as a single 1 dose of 5 mg or 2-4 mg/h as an infusion. According to Unger (57) the concentrations produced by cardiologists are probably 10,000 times the highest levels of endogenous glucagon which were about 700 pg/ml in the series of Lamiado et al. (24).

We used a relatively small dosage of glucagon

24 mg/74 h administered subcutaneously as a suspension in which the crystals are a combination of zinc protamine and glucagon. Tardag et al. (50) found in mongrel dogs weighing 4-30 kg, that this suspension in a dosage of 1 mg caused a gradual increase in plasma glucose from about 170 to 150 mg/100 ml 1 hour after the injection. The concentration remained at this level for the rest of the experimental period which was 6 hours. The FFA concentration declined to about 50% of the fasting level and returned slowly to the initial level. A serum insulin concentration of about twice the initial values was observed 1 hour after ZPG injection and maintained for at least 5 hours. Assan and Delamary (3) tested the same ZPG preparation in man as a single s.c. injection of 10 μ g/kg. This very small dosage yielded a plasma glucagon level of 500-1,000 pg/ml and a blood glucose level of 160 mg/100 ml in three subjects. The effects returned to the basal level within 3 hours and were considered to be not sustained enough to be of clinical importance. Kuhn (22) used 5-10 mg s.c. injections of ZPG in 11 patients with heart failure. The blood glucose level was found to be increased 92% after 3 hours and 43% after 6 hours. FFA decreased respectively to 35% and 29% of the initial values. In another series of 10 patients Kohn et al. (23) found a significant increase of 141% in blood glucose 60 min after a 5 mg injection of ZPG.

In the present study no si

blood glucose was achieved with ZPG in the 8 mg tid dosage. An explanation might be the co-existent hormonal changes due to AMI. The increase in plasma insulin concentration was significant in the small group of five patients 2 hours after the single injection of ZPG but not in the series of 22 patients treated with ZPG for 4 days. After the cessation of ZPG treatment no individual cases of hypoglycemia or hypoinsulinemia were seen on the following day. Köhn (22) reported severe hypoglycemic episodes in some patients possibly due to glycogen depletion in the liver after long-term treatment with glucagon.

The positive inotropic response of the heart on glucagon treatment was also statistically non-significant in our study when evaluated with heart rate and blood pressure as parameters. Glick (13) has suggested that if all the glucagon receptors of the heart are occupied by endogenous glucagon, any additional glucagon administered by injection would have little or no effect. The concomitant activation of adenylyl cyclase by catecholamines may also mask the action of glucagon in the heart. Levey and Epstein (26) found that the combined maximal effects of noradrenaline and glucagon on adenylyl cyclase in cat heart did not exceed the effect of noradrenaline on its own. A combination of isoproterenol and glucagon has however been found to produce a greater effect on cardiac output in patients than either drug alone (42).

To conclude it can be stated that in patients AMI a larger or more frequent dosage of PG must be used than that administered by us if marked changes in the inotropic state of the heart or in the metabolic or endocrine response due to the AMI are desired.

The usefulness of endogenous or exogenous hyperglycemia in AMI is still an open question as also is the importance of insulin administration. Glycolytic activity is near maximal already during normoglycemia and the possibilities of enhancing anaerobic glycolysis with insulin are limited (8, 34). Brachfeld (7) also states that an augmented anaerobic glycolysis is not always desirable since the increase in intracellular lactate and other acidic end products will result in a dangerous drop in intracellular pH. Most et al. (35) have recently demonstrated that glucose extraction of ischemic human myocardium did not rise with increased arterial glucose concentration.

Our findings suggest that in uncomplicated AMI

there is no need for treatment with either glucose, insulin or a combination of them if the patients are allowed to take regular meals. The condition of a patient with AMI complicated by cardiogenic shock or severe left heart failure may however be favourably influenced by the well tolerated injections of ZPG (22, 23) with glucagon (13, 27) or GIK infusions (2, 28). In treatment with I.v. infusions the rate and osmolality of infusions may be more important than the constituents of the solutions. This may also explain the varying results reported with various modifications of the Sodi-Pallares regimen (7). When an acute left heart failure in AMI is accompanied by reoccurring arrhythmias a functional improvement can be attained with glucagon without any increase in the incidence of dysrhythmias (13, 39). Treatment with I.v. glucagon infusion is preferred in critically ill AMI patients but in acute exacerbations of chronic heart failure subcutaneous injections of a long-acting glucagon preparation may be both a practical and effective method of treatment (22).

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ACID-BASE PATTERN OF CEREBROSPINAL FLUID AND ARTERIAL BLOOD IN BACTERIAL MENINGITIS AND IN ENCEPHALITIS

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Abstract The acid-base status of cerebrospinal fluid (CSF) and arterial blood has been investigated in sequential samples from 25 patients with bacterial meningitis and 8 with acute encephalitis. Patients with bacterial meningitis hyperventilated spontaneously throughout the first week to median values for arterial carbon dioxide tension of about 40 mmHg. The pH in CSF was less than 7.30 in 17 cases, within the normal range in 5 and above 7.34 in 7. On admission the following CSF sodium values were observed: lactate = 9 mmol/l, erythrocyte carbonic dehydratase = 14 mmol/l and pH = 7.23. The concentration of lactate and pH in CSF approached normal values this week. Patients with acute encephalitis also hyperventilated but pH values in CSF were close to normal. Only severe cases of encephalitis had elevated lactate concentrations in the CSF. Even in the presence of spontaneous hypocapnia artificial hyper-ventilation may be beneficial in cases where arterial carbon dioxide tension rises without simultaneous clinical improvement.

PATIENTS

The study comprises 33 patients admitted to the Department of Infectious Diseases Blegdams Hospital Copenhagen during March 1971-Jan 1972. Twenty-four patients had bacterial meningitis and eight had acute encephalitis.

Ten patients aged 18-28, had meningococcal meningitis. They all survived, and only one had residual damage (facial palsy). Artificial ventilation was not applied in these patients.

Nine patients, aged 46-77, had pneumococcal meningitis. Eight were critically ill on admission and three of them died: one from alternative endocarditis, one from obstruction by secretions in the airways and one unexpectedly without definite explanation. One patient had severe residual cerebral damage. Three of the patients were artificially ventilated for 1-4 days. Five patients—including the three latter—recovered without sequelae.

One patient had meningitis caused by *Streptococcus pyogenes* group A. He had severely impaired cerebral function but recovered completely. A 5-year-old boy with *Haemophilus influenzae* meningitis had convulsions and respiratory arrest 9 hours after admission. Despite artificial ventilation he remained brain-dead. Cardiac action ceased 3 days later. A 48-year-old man with a chronic suppurative otitis had *Proteus mirabilis* meningitis. He recovered and was operated on for otitis 12 days after admission, but 74 days later he presented signs of brain abscess and died few days later. Three patients had unquestionably purulent meningitis, but no bacteria were demonstrated. One with lymphatic leukemia and bulbar symptoms died from pneumonia after 33 days. One had destruction of the sella and primary insufficiency requiring substitution therapy. After 6 months his cerebral function had recovered fairly well. The third patient, who had hypogammaglobulinemia, recovered completely from the infection.

Eight patients had acute encephalitis of unknown etiology. Two patients had increasing antibody titer against herpes simplex virus, but brain biopsy not performed. Two patients had rather atypical disease with headache, drowsiness

Bacterial meningitis and acute encephalitis are serious diseases with a definite lethality. Bacterial meningitis can be treated with antibiotics. Additional "symptomatic" therapy to maintain adequate ventilation and circulation has often to be applied. In patients with encephalitis this symptomatic therapy may constitute a main remedy.

This study was part of a pathophysiological investigation in patients with bacterial meningitis and with encephalitis. A report on cerebral circulation and metabolism has been published previously (12). The present study comprises the acid-base status in cerebrospinal fluid (CSF) and arterial blood (AB). The aim of both studies has been to obtain a better basis for a symptomatic treatment of patients with bacterial meningitis and with encephalitis.

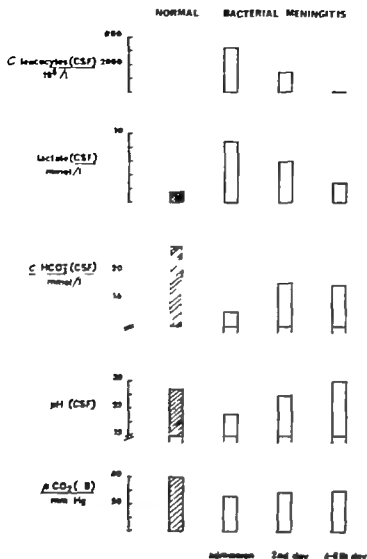


Fig 1 Median values of some acid-base variables in 25 patients with bacterial meningitis as compared to normal values. C = concentration, C = number concentration.

fusion. A single case was moderately severe with marked confusion and agitation. Five cases were very severe with coma, generalized epileptic seizures or attacks of decerebrate rigidity. In four of them artificial ventilation was necessary. Seven of eight patients recovered practically completely, whereas one had repetitive epileptic seizures progressing to brain death.

METHODS

The 25 patients with bacterial meningitis were investigated within 36 hours after admission and institution of antibiotic treatment. Twenty patients were reinvestigated on the second day and 14 had third investigation on the 3rd-8th day after admission. The eight patients with encephalitis were investigated 1-3 times within the first 16 days after onset of symptoms.

Samples of aB and CSF were drawn anaerobically in glass syringes. From 16 patients, in whom cerebral blood flow was studied simultaneously (12) CSF was

obtained by external puncture, whereas lumbar punctures were made in 8 others.

The following quantities were measured by means of equipment from RADIOMETER, Copenhagen. Carbon dioxide tension in aB pCO_2 (aB) and in CSF pCO_2 (CSF) was estimated using Severinghaus pCO_2 electrode type E5036/D616. Oxygen tension in aB pO_2 (aB) was estimated using a Clark oxygen electrode type E5046/D616. The pH(CSF) and pH(aB) were measured with Astrup thermostated microelectrode unit and a pH-meter (PHM 25) with a gas monitor (PHA 977). The pH(CSF) was estimated as described by Leven (6). The standardized concentration of hydrogen carbonate in plasma, $c(s)HCO_3^-$ (P) was assayed in fully oxygenated blood equilibrated at $pCO_2=40$ mmHg (4.5 kPa) (10). The $cHCO_3^-$ (CSF) was determined at pH 7.4 after addition of a measured excess of HCl using titration a selenoly (TTA 31 ABU 11 and TTT1).

Concentrations of lactate and pyruvate in CSF (lactate(CSF) and pyruvate(CSF)) were estimated by enzymatic methods (1). For these assays CSF was mixed

with an equal volume of ice-cold perchloric acid ($c=0.6 \text{ mol/l}$) immediately after sampling. A Zeiss PQM II spectrophotometer was used.

All assays were made in duplicate. The standard deviation of the mean of a duplicate determination (s) was calculated from the deviation of the two single values (No. of duplicates=20). The results were: $\text{pH}(\text{CSF})$ and $\text{pH}(\text{aB})$ $s=0.005$; $\text{pCO}_2(\text{CSF})$ and aB) $s=1.0 \text{ mmHg}$ (0.13 kPa); $\text{pO}_2(\text{aB})$ $s=2 \text{ mmHg}$ (0.26 kPa); $\text{lactate}(\text{CSF})$ $s=0.07 \text{ mmol/l}$; $\text{pyruvate}(\text{CSF})$ $s=0.005 \text{ mmol/l}$; $\text{cHCO}_3^-(\text{CSF})$ $s=1.13 \text{ mmol/l}$; $\text{c(HCO}_3^-(\text{P}))$ $s=1.3 \text{ mmol/l}$. pH , pCO_2 and pO_2 were determined at 37°C , but corrected to the rectal temperature (4, 16).

RESULTS

Meningitis. The 25 patients with bacterial meningitis are considered as one group in Fig. 1 which gives the median values. The material is separated according to etiology in Table 1 showing median values and ranges.

On admission the leucocyte number concentration of CSF and lactate(CSF) were increased to median values of $3.200 \times 10^6/\text{l}$ and 9 mmol/l respectively. The $\text{cHCO}_3^-(\text{CSF})$ was reduced to 14 mmol/l , and the $\text{pH}(\text{CSF})$ to 7.23. The pyruvate was increased, but considerably less than the lactate (CSF), so that the lactate/pyruvate ratio was ~ 3 times the normal value.

During the first week both the leucocyte number concentration of the CSF and lactate(CSF) decreased to median values of $83 \times 10^6/\text{l}$ and 2.85 mmol/l , respectively. The $\text{cHCO}_3^-(\text{CSF})$ increased already on the second day to 18.3 mmol/l , but remained subnormal throughout the study. The $\text{pH}(\text{CSF})$ gradually increased to approach the normal level within the first week.

Analysis of the aB on admission showed a hyperventilation with median values for $\text{pCO}_2(\text{aB})$ of 26 mmHg , for $\text{pH}(\text{aB})$ of 7.46 (normal range 7.35–7.42), and for $\text{c(HCO}_3^-(\text{P}))$ of 21.7 mmol/l (normal range 22.0–26.0 mmol/l). Six patients had subnormal $\text{pO}_2(\text{aB})$ values below 80 mmHg , the lowest value being 47 mmHg . None of them had a meningococcal infection.

The hyperventilation continued throughout the study with median pCO_2 values below 30 mmHg . The $\text{c(HCO}_3^-(\text{P}))$ was rapidly normalized, and the $\text{pH}(\text{aB})$ remained slightly elevated. None of the patients who had normal $\text{pO}_2(\text{aB})$ values on admission showed subnormal values later on. Five of the six patients with subnormal $\text{pO}_2(\text{aB})$ values on admission also had values below 80 mmHg later on.

A survey of the results from the individual patients showed that a prolonged clinical course coincided with prolonged CSF abnormalities as regards pleocytosis as well as lactate(CSF). The CSF findings were of no value in predicting the outcome of the infection.

Only minor differences in the CSF data distinguished the three main categories of bacterial meningitis (Table II). Patients with pneumococcal meningitis tended to have a more long-lasting elevation of lactate(CSF) than those with meningococcal meningitis, the latter also having a more favorable clinical course.

Encephalitis. As shown in Table II the lactate(CSF) was hardly increased above normal in mild cases. In the severe cases with decerebrate rigidity or convulsions lactate(CSF) was moderately increased to $\sim 6 \text{ mmol/l}$. The brain-dead patient had a lactate(CSF) elevation to 13.5 mmol/l . The pyruvate(CSF) and lactate/pyruvate ratio in the CSF were moderately elevated in most of the cases. The $\text{cHCO}_3^-(\text{CSF})$ was close to normal in mild cases but reduced to a varying degree in severe cases. The $\text{pH}(\text{CSF})$ values were almost normal with two exceptions. The brain-dead patient had marked acidosis. In case 6 the pH was clearly above normal.

Analysis of aB showed spontaneous hyperventilation with varying degree of $\text{pCO}_2(\text{aB})$ reduction, elevation of pH and usually normal $\text{pO}_2(\text{aB})$.

DISCUSSION

The typical finding in bacterial meningitis was a marked CSF lactacidosis and spontaneous hyperventilation in the acute stage. The CSF lactacidosis tended to subside rapidly whereas the hyperventilation continued. These findings were in agreement with previous studies by Montana and Perret (9) and by Blayo et al (1).

Patients with acute encephalitis had increased CSF lactate concentrations only in severe cases whereas almost all patients hyperventilated. We found no report on similar observations in patients with encephalitis.

These findings raise two questions: 1) What is the cause of the increased lactate concentration in CSF in meningitis and in encephalitis? 2) What is the cause of the sustained spontaneous hyperventilation in these patients?

Table I. Characteristic findings at different times in patients with bacterial meningitis of different etiologies

Both lumbar and cisternal CSF samples are included. Values of pH(CSF) and $p\text{CO}_2(\text{aB})$ from patients artificially ventilated are not included, except for cisternal CSF samples obtained during measurement of cerebral blood flow during artificial ventilation at $p\text{CO}_2(\text{aB})$ close to the level during spontaneous respiration.

C = concentration, C = number concentration, $\bar{x} = \text{mean}$, $s(x) = \text{SD of } x$.

Median values and ranges. No. of investigations given within parentheses.

	Normal values ($\bar{x} \pm 2s$)	Day of admission			Second day		
		Meningo- coccus AI (n)	Pneumo- coccus AI (n)	Other AI (n)	Meningo- coccus AI (n)	Pneumo- coccus AI (n)	Other AI (n)
leucocytes(CSF) ($10^6/\text{l}$)	0	2.91 (9) 523-33 000	4.850 (9) 37-8 064	4.000 (5) 766-18 500	1.179 (8) 14-4 140	1.640 (7) 80-3 000	1.6... (4) 16-4 290
lactate(CSF) (mmol/l)	1.54 0.97-1.6	8.79 (10) 6.03-21.20	8.35 (9) 3.88-40.30	11.45 (6) 4.40-28.70	4.11 (8) ... 13.80	7.26 (7) 4.14-1...70	5.49 (5) 4.54-17.60
HCO_3^- (CSF) (mmol/l)	3.6 1.8-25.4	16.8 (8) 7.6-18.6	15.0 (8) 9.7-20.0	13.0 (5) 11... 20.5	20.5 (8) 13.8-2...6	14.4 (7) 10.8-19.8	17.4 (5) 13.4-22.3
pH(CSF)	7.1	7.266 (7) 6.967-7.383	7.218 (7) 6.948-7.360	7.168 (3) 7.157-7.197	7.299 (8) 7...1-7.340	7.26 (5) 7.100-7.368	7.337 (5) 7.198-7.40
$p\text{CO}_2(\text{aB})$ (mmHg)	40	4 (9) 19-37	27 (8) 4-33	23 (5) 16-41	31 (8) 24-36	26 (5) 23-38	27 (5) 23-29

Table II. Cerebrospinal fluid and arterial blood quantities in patients with acute encephalitis

No.	Day after onset	Clinical situation	Artificial ventilation (d after onset)	Cerebrospinal fluid				pH	$p\text{CO}_2$ (mmHg)
				leucocytes ($10^6/\text{l}$)	lactate (mmol/l)	pyruvate (mmol/l)	lactate/pyruvate ratio		
2	10	Mild		88	1.17	0.173	10.0	-	-
7	4	Mild		15	-	-	-	-	-
	8	Mild		5	13	0.129	16.5	-	-
	8	Mild		-	1.57	0.097	16...	7.346	40
4	3	Moderately severe		33	2.00	0.115	17.4	-	-
	10	Mild		0	1.93	0.110	17.5	7.323	44
3	11	Severe		78	-	-	-	-	-
	16	Severe	16-18	1	3.31	0.190	17.4	-	-
5	4	Severe		104	4.40	0.154	28.5	-	-
	5	Severe		84	3.41	0.177	19.8	-	-
	9	Severe	5-10	136	6.05	0.479	12.7	-	-
8	2	Severe		15	2.69	0.211	12.7	7.423	40
	3	Severe		23	-	-	-	-	-
	6	Severe	3-7	-	5.02	0.375	13.4	-	-
11	5	Severe		4	3.60	0.137	15.2	-	-
	9	Mild		59	1.99	0.106	18.2	7.338	41
	11	Mild		46	1.61	0.09	17.7	7.291	41
1	13	Brain-dead	13-19	-	13.50	0.403	31.2	6.956	58

fourth-eighth day

Meningo- cocci M (n)	Pneumo- coccus M (n)	Other M (n)
48 (4)	31 (9)	1 020
8-134	21-1 408	32-1 836
66 (4)	4 64 (6)	5 05 (4)
1 48-4 76	2 30-8 46	2 76-9 04
23 8 (4)	17.2 (5)	17.5 (4)
21 6-26.2	1.3-25 6	14 0-18.0
7.354 (3)	7.349 (3)	7.296 (4)
7.348-7.365	7.299-7.367	7.200-7.373
32 (4)	28 (3)	30 (4)
29-33	25-35	4-39

arterial blood

pCO ₂ (mmHg)	pO ₂ (mmHg)	pH
31	94	-
36	-	-
34	109	-
32	87	7.46
32	77	-
36	102	7.42
28	-	-
25	-	-
29	73	7.475
33	57	-
33	126	7.455
30	81	7.453
32	143	-
33	149	-
34	93	7.380
36	86	-
37	55	-

The increased CSF lactate concentration

In patients with bacterial meningitis the increase of lactate(CSF) may be due to the presence of leucocytes and bacteria in the CSF as suggested by Montand and Perret (9). This suggestion was supported by the parallel decrease of the leucocyte and lactate concentrations of the CSF. It was further supported by previous *in vitro* studies on lactate production of leucocytes at rest and during phagocytosis (3-15). Other factors might contribute since lactate(CSF) was still increased at the time of the third sampling when the CSF leucocyte concentration was only slightly elevated in the majority of cases. Slow clearance of lactate from CSF (10) might be one possible explanation. Increased lactic acid production in the brain tissue due to inflammation might be another. Furthermore assuming that hyperventilation was caused by some other mechanism than CSF acidosis hyperventilation could by itself lead to increased lactate(CSF) (17).

In severe cases of encephalitis the lactate(CSF) increase could probably be explained in part by lactic acid production in inflammatory brain tissue and partly by the presence of decerebrate rigidity and convulsions (the latter of which may be accompanied by arterial hypotension (7)). Hypotension leads to an increased production of lactic acid in the brain. The CSF leucocyte concentrations were in every case of encephalitis so low that the leucocytes could hardly account for the presence of lactate.

Mechanism of hyperventilation

It is well documented that the pH(CSF) is a major factor in the regulation of respiration (9-11). The respiratory centers are extremely sensitive to pH changes. In patients with bacterial meningitis 29 pH measurements of lumbar CSF were carried out during spontaneous respiration with pCO₂(aB) below 40 mmHg. Seventeen cases showed a pH(CSF) below 7.30, five had a pH within the normal range (7.30-7.34) and seven had a pH above 7.34. Thus CSF acidosis could explain the hyperventilation in a substantial part of the observations.

However since the hyperventilation in a number of cases remains unexplained other mechanisms must be looked for—first of all the possibility of errors in pH(CSF) due to loss of CO₂ during sampling or measurement. Although care taken to avoid such errors, CO₂ loss

overestimation of the pH(CSF) may have occurred.

Because cisternal CSF samples were always taken during short-lasting artificial ventilation in connection with blood flow measurements only lumbar CSF samples obtained during spontaneous respiration were considered in establishing the relationship between $p\text{CO}_2(\text{aB})$ and pH(CSF). However Plum and Price (14) recently reported differences between cisternal and lumbar CSF samples with considerable individual variations but generally with lower pH and higher $p\text{CO}_2$ values in the lumbar samples. They concluded that lumbar CSF samples may provide unreliable information about cerebral acid-base status. Therefore our data cannot settle the question whether hyperventilation may be found in meningitis or encephalitis without local acidosis at the level of the respiratory centers.

Besides a CSF acidosis other factors could possibly stimulate respiration in these patients. Arterial hypoxia increases the ventilation when the $p\text{O}_2(\text{aB})$ is reduced to below 60 mmHg. Such low $p\text{O}_2(\text{aB})$ values were found in three patients only. Hypoxia was therefore an unlikely explanation in the majority of cases.

Raised intracranial pressure may be accompanied by hyperventilation. Since the lumbar CSF pressures were frequently about 400 mm H₂O in patients with bacterial meningitis this factor might be of importance.

By the possibility of a neurogenic mechanism should be mentioned. In cerebral apoplexy Lane et al (4) found a negative correlation between $p\text{CO}_2(\text{aB})$ and pH(CSF) in lumbar spinal fluid samples. They considered this finding to provide evidence that a "neurogenic mechanism" is responsible for hyperventilation. We did not find a similar correlation in patients with meningitis but five observations in encephalitis fitted a negative correlation ($p < 0.02$). The number of observations and the fact that they concerned lumbar CSF samples did not, however, allow extensive conclusions.

To sum up we were not able to demonstrate acidosis in lumbar CSF in every case of hyperventilation but this does not necessarily exclude local acidosis at the level of the respiratory centers. Increased intracranial pressure was a possible stimulus in meningitis and a "neurogenic mechanism" could not be ruled out in encephalitis.

THERAPY CONSIDERATIONS

Symptomatic therapy in bacterial meningitis and in encephalitis aims at maintaining ventilation and circulation at a level that offers the best chances of survival for the brain tissue.

Respiratory insufficiency may arise gradually from impairment of cough and/or swallowing reflexes in comatose or somnolent patients or from damage of the central mechanisms regulating respiration. In both cases manifest respiratory failure must be treated by means of artificial ventilation.

The $p\text{CO}_2(\text{aB})$ in patients with meningitis or encephalitis was frequently below 25 mmHg. Most of the patients did well and eventually recovered but in some cases an extremely bad cerebral condition was connected with $p\text{CO}_2$ values somewhat higher although still below the normal value of 40 mmHg. In such cases artificial respiration should be started without hesitation even if the patient does not show the above mentioned types of respiratory failure.

It is questionable at what level $p\text{CO}_2(\text{aB})$ should be kept during artificial ventilation. A low $p\text{CO}_2$ is probably beneficial in order to counteract CSF acidosis to reduce intracranial pressure and possibly to restore autoregulation (13). On the other hand extremely low $p\text{CO}_2$ levels may cause cerebral ischemia. A reasonable and safe compromise is probably a $p\text{CO}_2$ level of 30 mmHg.

The connection between CSF acidosis in meningitis or encephalitis and the regulation of cerebral blood flow has been discussed in another part of the present study (1). It should be mentioned here only that, in addition to a "relative hypercapnia," arterial hypertension and hypoxia appear dangerous factors in these patients.

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EPSTEIN-BARR VIRUS (EBV) INFECTIONS IN ARMY RECRUITS

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Abstract. Serum specimens collected from 477 recruits entering military service have been studied for fluorescent IgG antibodies to Epstein-Barr virus (EBV). Only 40 men (8.5%) were negative and 91.5% had antibodies to EBV. During the 8-11 months military service repeated serum specimens were taken from 35 antibody-negative men and at every infection of the upper respiratory tract. During this follow-up period 4 negative men (11.4%) had seroconversion. One of them had typical heterophil antibody-positive infectious mononucleosis, two had had tonsillitis 3 and 4 weeks before the specimens with seroconversion were taken, none of these three men had heterophil antibodies. The fourth man reported no illness. Serum specimens from all four recruits with seroconversion were studied also for fluorescent IgM gel-precipitating and complement-fixing antibodies to EBV. These tests confirmed the diagnosis of EBV infection.

Henle et al. (3) published in 1969 the first report of EBV as the etiological agent of infectious mononucleosis. Patients with heterophil antibody-positive infectious mononucleosis regularly developed antibodies to EBV which were clearly distinguished from heterophil antibodies. This disease is recognized as a common disease in older children and young adults, especially in college students (1, 9). Lehtinen (7) reported that among Finnish recruits and Vietnam veterans the rate of EBV seroconversions was 18.5-23.5/1 000 during the military service. Only one tenth were hospitalized for infectious mononucleosis whereas the remaining cases were more or less silent infections.

The present report describes the incidence of both silent EBV infections and the clinical infectious mononucleosis in two groups of army recruits altogether 477 men. After the initial serum survey the EBV antibody-negative men were studied serologically and clinically throughout the service period of 8-11 months.

SUBJECTS AND METHODS

Two groups of army recruits serving at the Military Training Center in Turku, Finland were chosen for this study. The first group entered military service in Oct. 1970 and the second in Feb. 1971 at the average age of 20 years. The total number of men studied was 477. A serum specimen was taken from each recruit entering military service, and the specimen retested for fluorescent IgG antibodies to EBV by the method of Henle and Henle (2) with modifications (6). Altogether 40 of the men (8.5%) were negative and thus considered to be susceptible to EBV infection. During the follow-up period a serum specimen was taken from 35 recruits at 3-month intervals during the whole military service, and at every infection of the upper respiratory tract. Five of the 40 men dropped out from the study soon after the first serum sample was taken because of furlough or transfer to another training center; the remaining 35 men were followed up throughout the whole military service (8-11 months). When seroconversion was detected among this group the clinical picture was clarified carefully. The serum specimens of the recruits with seroconversion were tested also for fluorescent, EBV-specific IgM antibodies by the method of Schmitz and Scherer (16) with modifications (10).

In this test the Burkitt cell line P3HR1 was used. The cells were kept at 37°C for 14 days before they were used for slides. The acetone-fixed slides were overlaid with serum dilutions, incubated in a moist chamber at 37°C for 3 hours. After washing with phosphate buffered saline (PBS) the slides were dried and overlaid with anti-human IgM conjugate (Wellcome) at 1:20 dilution and incubated again for 45 min. The slides were then washed again with PBS, mounted, provided with cover-glass and read off.

All sera from recruits with seroconversion were tested for gel-precipitating (GP) and complement fixing (CF) antibodies to EBV too. The antigen for GP and CF tests was made from P3HR1 cells after treatment with Tween 80 (11, 12). The heterophil antibodies were studied by the horse erythrocyte agglutination slide test (5). The positive sera were titrated in twofold dilution by means of slide agglutination.

Table 1 Results of EBV antibody titrations in serum from 4 recruits with seroconversion

FA=fluorescent antibodies

Pat. no	Weeks after beginning of military service	FA		CF	GP		Horse erythrocyte agglutination
		IgG	IgM		Lines	Grade	
I	0	<10	<10	<5	0	0	-
	8	<10	<10	<5	0	0	-
	23	<10	<10	<5	0	0	-
	32	640	320	10	1	1	+1/8
	34	640	320	10	2	1	+1/4
	42	160	<10	20	2	2	-
II	44	160	<10	20	2	2	-
	II	<10	<10	<5	0	0	-
	9	<10	<10	<5	0	0	-
	26	320	<10	10	1	1	-
	36	160	<10	10	1	1	-
	43	160	<10	10	1	1	-
3	0	<10	<10	<5	0	0	-
	9	80	40	10	1	1	-
	20	80	<10	20	2	2	-
	26	160	<10	20	2	2	-
	40	160	<10	20	2	2	-
	52	160	<10	20	2	2	-
4	0	<10	<10	<5	0	0	-
	9	40	<10	<5	0	0	-
	10	40	<10	<5	1	1	-
	19	80	<10	<5	1	1	-
	26	40	<10	<5	1	1	-
	36	40	<10	<5	2	2	-
	43	40	<10	<5	2	2	-

RESULTS

In the study population of 477 men 91.5% had fluorescent IgG antibodies to EBV. In the follow-up group of 35 men without antibodies 4 (11.4%) opened antibodies to EBV during the military service. One of whom had a typical infectious mononucleosis and 3 a more or less atypical clinical picture.

CASE REPORTS

Case 1

A 19-year-old farmer living in the country was negative for EBV antibodies on April 6th 1971. In the 23rd week of military service. He visited the doctor on June 7th at the 31st week of military service because of a headache, chest pain and slight fever of 38°C and was admitted to hospital. ESR was 3 mm/h, Hb 160 g/l, serum creatinine 116 µmol/L. Urinalysis and chest X-ray were normal. On June 11th, after 32 weeks of service, WBC was 6700, bands 0.02, segs 0.25, lymphs 0.65, monos 0.08 and the horse erythrocyte agglutination was negative. The serum revealed fluorescent IgG and IgM antibodies and both CF and GP antibodies to EBV (Table 1). At physical examination he had a slight swelling under the ear and at the corners of the jaw on the left side and enlarged cervical glands on both sides. Tonsils were red and enlarged

with white spots. The left side of the pharynx was swollen and peritonsillar abscess was suspected, but the consulting otolaryngologist excluded this and confirmed the diagnosis of infectious mononucleosis. The patient was sent to his unit and bed rest was ordered. On June 14th ESR was 18. The patient recovered rapidly without any complications. The next serum specimen was taken on June 24th in the 34th week of service, and revealed the same antibody titers to EBV except that one more GP line had appeared and the horse erythrocyte agglutination titer had dropped from 8 to 4. On Aug. 25th, in the 42nd week, the IgG antibody titer had decreased from 640 to 160 and neither IgM antibodies to EBV nor heterophil antibodies were detectable. The CF titer had increased as also had the intensity of GP reaction. In the 44th week, just before the end of military service, his titers remained constant.

Case 2

A 19-year-old garage worker living in town was negative for EBV antibodies on April 19th 1971 after 9 weeks of military service. He was sent to another training center and had been admitted to the local hospital on July 4th for 3 days because of diarrhea, vomiting and abdominal pain. At physical examination the abdomen was diffusely tender on palpation. ESR was 3 mm/h and temperature 37.3°C. He recovered completely after 2 days. The diagnosis of acute gastroenteritis was made. The next specimen taken on Aug.

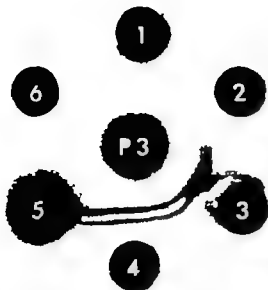


Fig. 1 Development of GP antibodies to EBV in serum from patient 3. 1=pre-illness serum, taken at entry to military service. 2=in 9th week, 3=in 20th week, 4=in 26th week, 5=a control serum with weak activity. 6=PBS, P3=EBV antigen from the Berrion line P3HR-1.

26th, in the 26th week of service, was positive for IgG CF and GP antibodies to EBV (Table 1). No IgM antibodies to EBV or heterophil antibodies were detectable. It is possible that the EBV infection was sub-clinical or with very slight symptoms and the gastroenteritis was caused by some other agent.

Case 3

A 20-year-old welder living in town was negative for EBV antibodies on admission into the service on Feb. 15th, 1971. The second specimen taken on April 19th, in the 9th week of service, was positive for EBV IgG IgM, CF and GP antibodies, but not for heterophil antibodies (Table 1). On March 15th he had had tonsillitis with 39°C fever for 1 day and was admitted to hospital. ESR was 34 mm/h on the next day and 4 mm/h 3 days later. He was treated with phenoxymethylpenicillin 800 000 U every 6 hours by mouth, and was sent to his unit after 5 days. It is highly probable that this infection had been caused by EBV because the late convalescent serum taken 4 weeks after the illness still contained IgM antibodies to EBV. The third specimen taken on Aug. 26th, in the 20th week of service did not contain IgM antibodies but the CF and GP antibody reactions were stronger than in the earlier specimens (Fig. 1). The specimens of the 40th and 52nd week revealed the same antibody levels.

Case 4

A 19-year-old merchant, living in town, was negative for EBV antibodies at the beginning of military service on Feb. 15th, 1971. The second specimen taken on

April 19th, after 9 weeks of service, was positive for IgG antibodies to EBV but negative for heterophil antibodies. No IgM or other types of EBV antibodies were detectable. On March 25th he had had tonsillitis and was admitted to the garrison hospital for 4 days. Temperature was 38-39°C for 3 days and ESR 25 mm/h. He was treated with phenoxymethylpenicillin 800 000 U every 6 hours by mouth. He recovered rapidly and was sent to his unit. In the serum specimens taken on April 28th in the 10th week, one week later than the previous specimen, there were already GP antibodies. He did not develop CF antibodies to EBV but the GP reactions became still stronger up to the 36th week (Table 1).

All these recruits had a clear seroconversion not only for IgG but also for GP antibodies which are sometimes clearly demonstrable (Fig. 1). Only three men had a seroconversion for CF antibodies to EBV. The micro GP technique has also earlier been demonstrated to be more sensitive than the CF technique for EBV antibody detection when the crude antigen preparation is used (11). Both the GP and CF antibodies developed slowly which has some advantages in this type of follow-up. These antibodies are in general detectable 4-6 weeks after the onset of disease which makes it possible to determine the time of the acute phase of infection retrospectively. The IgM antibodies to EBV were detectable in two patients (nos. 1 and 3) (Table 1). In the two others the IgM antibodies had already disappeared. Only one patient (no. 1) who had the typical infectious mononucleosis had also heterophil antibodies. The serum specimens from all 477 recruits were titrated for fluorescent IgG antibodies to EBV. Initially 437 men, 91.5% were positive for EBV antibodies. Since most of the recruits are about 20 years old also the majority of the negative recruits belonged to this age group. The small number of recruits younger or older than 20 did not enable one to calculate statistically the rate of EBV positiveness in the other age groups. The distribution of antibody titres of the whole population of recruits at entry into military service is shown in Fig. 2. The titres most commonly varied between 80 and 320. 77.5% of the titres falling within this range. The immunological profile (13) of the whole material of recruits is shown in Fig. 3 and the median titer 174 is calculated from the figure.

In earlier studies on EBV antibodies it has been reported that the prevalence of antibodies is highest in the low socio-economic groups. In this

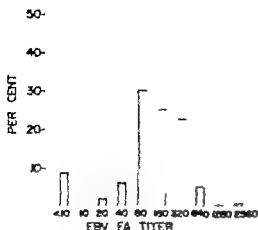


Fig. 2. Distribution of fluorescent IgG antibody titers to EBV in serum from 477 army recruits of average age 20 years.

study the recruits were divided into three social groups according to Rauhaala (15). Because very few of them belonged to group I this and group II have been combined as the higher socio-economic level group III forming the lowest level (14). In this study there was some difference in the percentage of EBV negative recruits in the higher and in the lower social groups. The higher group had more negative men than the lower but the difference was not statistically significant. The material was also classified according to the place of residence but there was no significant difference between the rural and urban recruits as far as prevalence of antibodies is concerned. The recruits were divided into three groups according to education in the army as men, non-commissioned officers and officers; no statistical differences were noted.

DISCUSSION

In the present study EBV infections were followed during the military service in two groups of army recruits. These infections were less common than expected. Taking into consideration that 4 (11.4%) of 35 men who were susceptible to this infection fell ill within 11 months it was however not especially rare among the susceptibles. Only one recruit had a typical infectious mononucleosis with heterophil and IgM antibodies to EBV. Two recruits had a tonsillitis shortly after entering military service when results from the antibody titrations of the first serum specimens were not

yet available. Thus we lack the acute phase specimens from those two. The late convalescent serum specimens taken 3–4 weeks after the onset of disease are heterophil antibody-negative. One of these two had IgM antibodies to EBV the other was already negative. It is highly probable from the clinical data and from the EBV antibody response that these infections were caused by EBV. The only infection of the fourth recruit with a seroconversion was a gastroenteritis 7 weeks before the EBV-positive serum specimen was taken. This sample was also negative for heterophil antibodies. It is possible that this disease was caused by EBV but it is perhaps more probable that the EBV infection was sub-clinical.

Lehane (7) has reported that the inapparent infections are ten times more frequent than the apparent. All of our four patients with seroconversion developed GP antibodies but only three had CF antibodies to EBV. These two methods are convenient in this type of follow-up, because the GP and CF antibodies develop slowly. The GP and CF antibodies are detectable usually 4–6 weeks after the onset of disease and keep rising up to about 16 weeks. In some cases as reported earlier (11), by these methods combined with the disappearance of IgM antibodies to EBV it is possible to determine the approximate time of the acute phase of the disease. Thus the symptoms of clinical infections can be studied retrospectively.

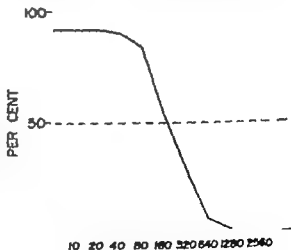


Fig. 3. Immunological profile of fluorescent IgG antibody titers to EBV in serum specimens from 477 army recruits. The crossing of the 50% level and the immunological profile indicates the median titer 174.

ly. In the case of an EBV infection this might be valuable because the symptoms are variable from subclinical infections to clinical infectious mononucleosis.

EBV infections do not seem to be especially common among Finnish army recruits as a whole since about 9% are already infected at that age. As high a prevalence as this is in the comparable age group has been reported from Sweden (17) from the USA (Chicago and Alaska) (18) and from Japan (4). In Lehtinen's study (7) the prevalence of EBV antibodies was 86% and the rate of sero-conversions during military service was about three times higher than in our material (4/77 (0.84%). However Niederman et al. (8) have reported that the total incidence of EBV infections per year among susceptible college students is 11.1% the same as in the present study (11.4% (4/35). They also reported that the inapparent-to-apparent case ratio was 1:2 as compared to 1:3 in this study. There are reports in which people with low socio-economic background have had antibodies to EBV more often than those from high social groups (3, 9). There was a similar tendency in the present findings but the difference was not significant.

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CASE REPORT

The patient is a woman born in 1918. Her father died at the age of 81 in a leukemic disease and brother at the age of 45 of renal carcinoma. She underwent two operations on the spinal column in 1946 and 1949 because of scoliosis and herniated lumbar disc respectively. Polyps in the rectum were locally resected in 1944 and 1945. In 1968 she was unsuccessfully subjected to dietary treatment of obesity during a 4-week period as in-patient. Because of an urtic plasma protein analysis was performed in 1969. The analysis was done by the Clinical Laboratory of the University Hospital of Uppsala and revealed clearly subnormal levels of IgG and IgM with IgA just below the normal range. The following values were reported: IgG 4.7, IgA 0.8 and IgM 0.1 g/l. No M component was found. Unfortunately no test for Bence Jones proteinuria was performed.

In 1970 a carcinoma of the transverse colon was detected and colectomy was successfully performed. No evidence of metastases was found at the operation and the ileostomy has been functioning well since then. Her weight has remained constant.

In Jan 1974 she came as out-patient to the department of Internal Medicine of Avesta Hospital complaining of moderate pain in the back and the legs. The physical examination revealed little except the previously noted obesity. BP was 160/90. Laboratory investigations yielded the following results: ESR 3 mm/hour, Hb 114 g/100 ml, erythrocytes 4 400 000/mm³, leucocytes 5300/mm³, with normal differential, platelets 178 000/mm³. Plasma protein analysis was performed by the Clinical Laboratory of the University Hospital of Malmö and again revealed the same pattern of immunoglobulin deficiency as in 1969. The following values were reported: IgG 4, IgA 1.0 and IgM < 0.2 g/l. No component or any other protein abnormality was

Urine electrophoresis performed by the same laboratory revealed 175 mg% of Bence Jones protein of kappa type.

Because of the finding of light chains in the urine the patient was admitted to the hospital as in-patient with the presumptive diagnosis of light chain disease. A complete roentgenological examination of the axial skeleton as well as of the long bones of the extremities was performed without any unequivocal evidence of any destructions. Because of slight irregularity of the right seventh rib, an extra X-ray was taken of this area. No destruction was found however. Serum creatinine was 1.09 mg/100 ml, serum calcium 5.0 mEq/l. Liver function tests were all normal. Bone marrow from the sternum was sent to I. Granberg at Huddinge Hospital who reported completely normal findings without any increase in the number of plasma cells. The patient was discharged with the presumptive diagnosis of idiopathic, benign light chain disease.

DISCUSSION

Immunoglobulin deficiency without an M component in the plasma combined with Bence Jones proteinuria is characteristic of the majority of

cases of light chain disease (4). In our patient the pattern of immunoglobulin deficiency has remained more or less constant over a 5-year period. Bence Jones proteinuria has been detected at the beginning of 1974. No test for light chains in the urine had been performed previously. We have found no other clinical evidence of myelomatosis in this patient. She continues to be in a non-deteriorating clinical condition with slight symptoms attributable to her overweight.

This patient may of course after an initial period of many years of a steady level of immunoglobulin abnormalities later develop progressive light chain proteinuria together with the clinical picture of myelomatosis. She may still be in a dormant, premyelomatous stage of an eventually malignant plasmacellular dyscrasia.

Another possibility is that she may have a solitary plasmacytoma that cannot be clinically localized. Hobbs (1) has reported that the hypogammaglobulinemia of a patient with a solitary plasmacytoma of the head of the femur rose to normal levels after the tumor had been removed and remained normal two years later. He is of the opinion that a solitary plasmacytoma may release a humoral factor which can depress the protein production of the normal plasma cells. Theoretically therefore even in solitary plasmacytomas not detectable by ordinary clinical methods, signs of disturbed immunoglobulin metabolism could be evident. In our case we would have to postulate a light-chain-producing, solitary plasmacytoma.

A third and equally intriguing possibility of course, is that she may have a truly idiopathic light chain dyscrasia. Only continued follow-up may give us the chance to determine whether one of these three alternative hypotheses will prove to be correct or whether another type of disease associated with Bence Jones proteinuria will ultimately develop.

In our present opinion however the fact that the plasma protein abnormalities of our patient have remained more or less constant over a 4-year period speaks in favour of a benign plasmacellular dyscrasia. In a case of light chain disease it would be exceedingly unusual not to have definite progression of abnormalities within such a long period. We should therefore like to suggest that our patient has a form of benign light chain dyscrasia, a hitherto unrecognized type of benign gammopathy.

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Announcements

An International postgraduate course on clinical cardiology will be held in Davos Switzerland, Feb 16-21 1975 Organized by Dr H Pitt, Associate Professor of Medicine The Johns Hopkins Hospital Baltimore Maryland USA in cooperation with lecturers from the USA and Europe. Sponsored by the American Heart Association

Subject. Diagnosis and therapy of angina pectoris and myocardial infarction.

Further information and registration forms (European participants): Prof Dr P Lichtlen Abteilung für Kardiologie Department für Innere Medizin Medizinische Hochschule Hannover D-3000 Hannover BRD (Participants from overseas) Dr B Pitt, Associate Professor of Medicine Division of Cardiology The Johns Hopkins Hospital Baltimore Md USA Number of participants is limited Closing date for registration Dec 31 1974

International Symposium on Intensive Therapy total parenteral alimentation, will be held in Rome Italy May 30-June 2, 1975

Organizing secretariat International Symposium on Intensive Therapy New Media Via Giovanni Battista Martini 6 I-00198 Rome Italy

The Sixth International Congress of Nephrology will be held at the Palazzo dei Congressi in Florence Italy June 8-12, 1975

Scientific Program Committee Nephrology Dialysis Dept. S Orsola University Hospital Via Massarenti 9 I-40138 Bologna, Italy

Social Program Committee Institute of Urology University of Florence Viale Pieraccini 18 I 50139 Firenze Italy

THANKS TO OUR REFEREES

During the last two years when I have been Chief Editor of this journal, the referee system has been used systematically. The Editors want to express their gratitude towards the many colleagues who have given time and thought to this task. We hope that we shall have the collaboration of these and other specialists within different fields also for future volumes of our journal. Through their efforts many faults have been discovered and many obscure points clarified.

It is our hope that the referees will continue to help us to raise the standard of the Acta and we believe that this journal—and thus its readers—have profited greatly from the suggestions and positive criticism.

Anonymity has been a condition for unbiased work as a referee. A list of their names gives us a chance to remember their efforts without unmasking the individual.

P. Bechgaard, K. Brøchner Mortensen, V. Faber, J. Hers Thaysen, K. Iversen, Sv. Aa. Killmann, V. Fosborg Petersen, J. Poulsen, M. Schwartz, A. Tybjaerg Hansen, N. Tygstrup, P. Halonen, W. Kar-

painen, B.-A. Lamberg, E. A. Nikkilä, O. Wegelius, E. M. Blegen, O. J. Broch, E. Enger, O. Storstein, S. Almqvist, U. T. Axelsson, S. Å. Belfrage, U. Bengtsson, S. E. Bengtitz, J. Bergström, Å. Bertler, G. Böörck, P. Björntorp, L.-O. Brandt, L. A. Carlson, J. Dymling, S. Efendić, H. Ehasch, L. Engstedt, L. R. Erhardt, S. Eriksson, H. Forsman, L. Garby, S. Gardell, G. Gahrton, A. Gustafsson, K. Gydell, K. Hall, L. Å. Hansson, R. Hed, S. Hofvendahl, C. Höglman, B. Hökfelt, D. Ingvar, B. Johansson, A. Killander, C. B. Laurell, T. Leinhardt, S. Lindstedt, J. E. Lindsten, J. G. Ljunggren, T. Lundberg, B. Lundh, T. Lundman, R. Luft, G. Magnusson, H. Malmros, V. Mott, H. Möller, H. Nilsson, I. M. Nilsson, B. Norrén, E. Orskov, B. Pernow, H. Reichard, P. Reizenstein, A. Rosén, H. Scherstén, H. Sjöberg, A. Sjögren, F. Sjöqvist, N. Söderström, N. Törnblom, L. Werld, W. O. Wester, L. W. Wilhelmsen.

Acta Medica Scandinavica thanks its many competent referees and wishes them as well as our authors and readers a happy New Year 1975.

J. n G. Waldenström

ACETYSALICYLIC ACID INTOLERANCE

Lack of N-acetylsalicylic Acid Specific Skin-sensitizing Antibodies in the Serum of Intolerant Individuals

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Abstract Twenty-seven ASA-intolerant individuals were investigated. Twenty-six of them suffered from chronic bronchial asthma, one from urticaria. All reacted to the ingestion of therapeutic dose of ASA with more or less violent signs and symptoms resembling a systemic allergic (anaphylactic) reaction of the immediate type. Seventy percent of them reacted in the same way to one or more chemically unrelated drugs. Seventeen of these patients were skin-tested with N-salicyloyl- and N-acetylsalicyloyl-PLL without the appearance of immediate type reactions. Furthermore, the sera of these patients were passively transferred to rhesus monkeys. The intradermally sensitized monkeys were challenged by or intradermal injection of N-salicyloyl-BSA or N-acetylsalicyloyl-PLL or by oral administration of ASAN or ASA. No positive reaction was observed. Intracutaneous sensitization of rhesus monkeys with the sera of individuals allergic to Timothy grass pollen revealed strong reactions of the immediate type following challenge with Timothy grass pollen extract. The IgE levels and the concentrations of the other immunoglobulins of the ASA-intolerant individuals were found to be within the normal range. Thus, homocytotropic antibodies with N-salicyloyl or N-acetylsalicyloyl specificity could not be detected in the ASA-intolerant individuals. These results, the frequency of intolerance to other drugs, the familial occurrence of ASA intolerance and the demonstration of "latent" ASA intolerance in about 20% of chronic asthmatics indicate that the clinical picture of the syndrome is not sufficient criterion of its allergic origin. In our opinion the syndrome is caused by direct (i.e. not antibody mediated) liberation of histamine and other mediators by the offending drugs due to an "inborn error of metabolism" in intolerant individuals. The syndrome should be properly denoted as drug-induced anaphylactoid syndrome.

Adverse reactions to acetylsalicylic acid (ASA) resulting in smothering severe asthma attacks, angioedema, urticaria, fall of the BP and in few cases lethal shock (13, 18, 19, 28, 33, 53, 54) have been known for more than 60 years (23, 45). The major

symptoms of these untoward reactions to ASA resemble those of an allergic disorder of the immediate type. Therefore it has been assumed that individuals showing such reactions after intake of therapeutic doses of ASA are allergic to it. This assumption became understandable by the detection of a potentially immunogenic impurity present in low amounts in commercially available ASA preparations, namely acetylsalicylic acid anhydride (ASAN) (12).

Skin testing of ASA-intolerant individuals usually fails to give positive results (24, 40, 56, 59). This and the fact that a great number of ASA-intolerant individuals show intolerance to other chemically unrelated drugs too suggest the involvement of non-immunological rather than an allergic pathogenesis of the majority of adverse reactions to ASA (46). However, some cases with adverse reactions to ASA showing positive skin tests have been reported (12, 36, 46). Girard et al. (25) described 4 cases with a serum sickness type of reaction to ASA, two of whom showed an Arthus-type skin test. Lazary et al. (36) advanced the hypothesis that urticaria but not the other adverse reactions to ASA are of allergic origin.

In this paper evidence will be presented that in ASA-intolerant individuals no homocytotropic antibodies (3) are detectable either by skin testing with N-acetylsalicyloyl-poly L-lysine (PLL) (12) or by passive transfer of their serum into rhesus monkeys (34, 35).

MATERIAL AND METHODS

Antigens

Antigens used for skin testing of ASA-intolerant individuals and for that of passively sensitized rhesus monkeys

Table 1 Clinical data of ASA-intolerant patients and symptoms after ingestion of ASA

A=asthma, R=allergic rhinitis, U=urticaria, AE=angioedema, Ec=eczema, Mi=migraine, P=nasal polyps, N=nausea, V=vomiting, UC=unconsciousness, =severe, ss=very severe, n.d.=not done

Pat. no.	Age (y)	Sex	Original complaint	Reaction after ingestion of ASA	Skin test*	Intolerances or allergies against other drugs
I	29	♀		U	Neg.	—
II	45	♀	U, Mi	AE, V	Neg.	—
3	76	♀	Ec, Mi	U, R, V _{ss}	Neg.	Sansert®: N, UC
4	59	♀	A, Mi	As, V	n.d.	Quindine V, A
5	57	♂	A	As, V	n.d.	—
6	44	♂	A, R	As, V	n.d.	Indomethacin, V, A
7	III	♀	A, R, P	A, V	n.d.	Indomethacin, Hygroton®: V, A
8	50	♀	A, R, Ec	A, V _{ss}	n.d.	—
9	46	♀	A, R	A, AE, V _{ss} , U	Neg.	Barbiturates V, U
10	67	♀	A	AE	Neg.	—
11	51	♂	A, R, P	A, AE, V	n.d.	Sulphonamides, A, AE, V
12	53	♀	A, U	A, U, V _{ss}	Neg.	Penicillin: A, U, V _{ss}
13	47	♀	A, R, Mi	A, V	n.d.	Penicillin: U
14	46	♀	A, R	A, V	n.d.	Penicillin, A
15	80	♀	A	As, V	Neg.	Penicillin, UC, low BP
16	52	♀	A, R	As, V	n.d.	Saridon®: As, V
17	55	♀	A, R	As, V	Neg.	Sulphonamides, quinine: A, V, vertigo
18	54	♀	A	A, V _{ss} , vertigo	Neg.	Indomethacin, A, V, vertigo
19	48	♀	A, R	As, V _{ss}	Neg.	Sulphonamides: A, V _{ss} ; penicillin, U
20	59	♀	A, AE, Mi	As, AE, V	Neg.	Indomethacin, Reciparvin®: penicillin, erythromycin, iodine, quinine, Lasix® quinine (also peas, apples, onions, grape fruit), A, AE, V _{ss}
21	60	♀	A, R, U, AE	A, R, U, AE, V	n.d.	Hygroton, Salures, Isotoxyl®: quinine, sulphonamides, A, R, U, AE, V
22	65	♀	A	Fixed xanthema	Neg.	—
23	53	♀	A, R	A, AE, V	Neg.	—
24	25	♂	A, U, Ec	As, N, V	Neg.	—
25	50	♀	A	A, AE, V, shock	n.d.	Novocain®: Xylocain®: shock
26	50	♂	A	A, AE, V	Neg.	Saridon®: V, AE
27	79	♀	Hay fever	AE, UC	n.d.	Novocain, Xylocain®: UC

With N-acetylsalicyloyl/salicyloyl-PLL. Thermal.

conjugates of ASA or salicylic acid with PLL ($n=40$) or with bovine serum albumin (BSA). 1) N-acetylsalicyloyl/salicyloyl-PLL in which 25% of L-amino groups of the PLL were reacted with N-acetylsalicyloyl-hydroxysuccinimide ester. The residual L-amino groups were succinylated. 2) N-acetylsalicyloyl/salicyloyl-PLL in which 50% of the L-amino groups were salicylated by reaction of PLL with ASA chloride. 3) BSA was reacted with salicylic acid nitrophenyl ester resulting in conjugate which contained 13–14 N-salicyloyl residues/mole BSA. The antigen solutions were sterilized by filtration and lyophilized in sterile ampoules.

The antigens used in skin testing of ASA-intolerant individuals and in the passive transfer studies have been prepared and chemically characterized by Dr E. Schnabel, Biochemical Laboratory, Bayer AG, Wuppertal (48). The antigens employed were assayed for their anaphylactogenic activity in guinea pigs which were sensitized intradermally with ASAN dissolved in dimethylsulphoxide according to De Weck (12). All animals sensitized on days 1, 3, 6, 9 and 13 with 1 mg ASAN exhibited systemic anaphylactic reactions after i.v. challenge with the

N-acetylsalicyloyl or N-salicyloyl conjugates (antigens 1 and 2).

Skin testing of ASA-intolerant individuals

Skin tests were carried out by intradermal injection of 0.02 ml of a 1% solution of antigens 1 and 2.

Passive transfer of sera of ASA-intolerant individuals to rhesus monkeys

Rhesus monkeys (*Macaca mulatta*) weighing 2.5–3 kg were used for passive transfer experiments. To visualize the antigen-antibody reaction in the passively sensitized skin after antigen challenge the animals received 1–1.5 ml/kg of a 0.5% Evans blue solution in buffered saline either simultaneously with the antigen or 15–20 min after intradermal or oral application of antigens or haptens according to Leyton et al. (34, 35). The sera tested in these transfer experiments were filtrated through 0.22 μ Millipore filters and stored in portions of 0.5–1 ml in sterile ampoules at -20°C . Trials for the presence of tissue-sensitizing antibodies in the sera of ASA-intolerant individuals were performed according to the following experimental records.

Table II Synopsis of clinical data of ASA-intolerant patients

	Reference no.											
	44	17	20	56	43	27	46	4	45	16	22	23
	%	%	%	n %	%	%	%	%	%	%	%	%
Males	22/35	17/34	17/38	26/31	1/43	32/40	78/43	11/46	20/43	1/40	1/43	1/33
Females	40/65	33/66	28/62	57/69	11/43	57/60	104/57	12/52	26/57	18/60	4/67	4/67
Total	112	50	45	83	28	81	182	23	46	30	16	16
Age >30 y	41/66	-	32/71	46/55	23/82	69/85	136/75	13/57	34/74	-	14/34	14/34
Chronic asthma	43/69	46/92	45/100	78/94	28/100	81/100	-	23/100	-	4/80	-	-
Nasal polyps	21/34	33/66	21/47	33/40	9/32	47/58	9/51	11/48	15/33	30/100	-	-
Reactions after ingestion of ASA												
Asthma	58/61	33/63	45/100	73/83	24/86	80/99	154/85	20/87	15/33	12/40	2/6	-
Asthma and/or urticaria and/or angioedema	5/8	-	-	1/1	-	1/1	10/5	3/13	-	5/17	14/39	-
Angioedema and/or urticaria	13/1	13/25	-	10/11	4/14	-	18/10	-	20/47	-	20/55	-
Others	11/10	6/1	-	4/5	-	-	-	11/4	13/43	-	-	-
Intolerances or allergies to other drugs	19/31	18/36	13/29	13/15	17/61	-	42/23	2/9	14/30	1/70	1/58	-
Range 30-50 years	Including asthma+vasomotor rhinitis and asthma+abdominal cramps											
>35 years	Including seasonal and/or environmental inhalants. Wheezing, dyspnoea											
Range 30-40 years												

Experiment I 0.05 ml of each of 17 sera of ASA-intolerant patients were injected intradermally and the injection sites marked. After an interval of 74 hours 10 mg of N-acetylsalicyl-BSA or 10 mg of N-acetylsalicyl-PLL were applied together with Evans blue A control. 0.05 ml of normal human serum was applied intradermally immediately prior to the injection of Evans blue. Histamine dihydrochloride was given intradermally in concentrations of 10, 20 and 30 µg/ml calculated as histamine base. The animals were kept under observation for 6 hours after antigen challenge.

Experiment II Passive transfer of patient serum was carried out as in experiment I. Antigen challenge was performed 24 hours later by intradermal injection of 0.05 ml of 1% solutions of N-acetylsalicyl-BSA or N-acetylsalicyl-PLL into the marked sensitized skin areas. Normal human serum was used as the negative control. The antigens used for challenge did not induce reactions when injected into non-sensitized areas of the skin. Histamine was given in the same concentrations as described. Evans blue was applied 15-20 min after intradermal antigen challenge. The animals were observed for 6 hours.

Experiment III The experimental procedure and the controls were the same as in experiment I and II. Antigen challenge was performed 74 hours after skin sensitization by oral application of 30 mg ASAN to one animal and 300 mg ASA to another. Evans blue was injected 15-20 min after ingestion of ASAN or ASA. The animals were observed for 7-8 hours.

T demonstrates the skin-sensitizing properties of human

homocytotropic antibodies in primates. 0.05 ml of the serum of two individuals sensitive to Timothy grass pollen were injected into the skin of rhesus monkeys. The serum was applied in original concentration and diluted 1:10 and 1:100 with buffered saline. 0.4 ml Timothy pollen extract were given together with 0.5% Evans blue (1 µg/kg) 24 hours after sensitization of the skin. Normal human serum and histamine were applied as described. The serum of Timothy pollen sensitive patient and the Timothy pollen extract (V-trian 81153) were gifts from Prof. Dr G. Johansson, Uppsala.

Determination of immunoglobulins

The concentrations of IgG, IgA, IgM and IgD in the sera of 19 ASA-intolerant patients and 20 healthy human individuals were estimated by the radial immunodiffusion technique using Immoplates® (Hyland-Travenol GmbH, München). IgE was determined by radioimmunoassay test (Phadebas® test, Pharmacia, Uppsala). Employing the standard according to the British Research Standard for Human Serum Immunoglobulin E, 68/341 the IgE concentrations were calculated on the basis of 2 ng/U.

RESULTS

The present study is based on observations for several years in 77 cases of ASA-intolerant individuals (22 females, 5 males) who received medical treatment of the diseases listed in Table I. Of the 27

Table III *Personal and clinical data of ASA intolerant individuals and their reactions after ingestion of ASA*

	%	
Original complaints		
Asthma (with or without rhinitis)	19	70.4
Asthma, urticaria and/or angioedema	4	14.8
Others	4	14.8
Nasal polyps	2	7.4
Intolerances or allergies to other drugs	19	70.4
Reactions after ingestion of ASA		
Asthma (with or without vomiting)	13	48.2
Asthma, urticaria and/or angioedema	8	29.6
Angioedema and/or urticaria	5	18.5
Others	1	3.7
Vomiting	13	85.2
Reactions with symptoms of the original complaints	21	77.8
Reaction of asthmatics with asthma	21	91.3
Reactions with other symptoms than original complaints	6	22
Reaction of asthmatic with other symptoms than asthma	2	8.7

patients III were above 30 years of age. Intolerance reactions to ASA and a variety of other drugs structurally unrelated to ASA were explored by thorough investigation of the personal history of these patients and direct observation of the signs and symptoms occurring after intake of ASA or other drugs listed in Table I. No provocation tests were performed because these are dangerous.

The majority of our ASA-intolerant patients are female (81.5%) and include predominantly middle-

aged and elderly individuals (85.2%). About 85% of the patients suffered from chronic asthma bronchiale. In contrast to a variety of reports in the relevant literature (Table II) only 7.4% of our patients exhibited nasal polyposis. In about 85% of our patients vomiting was one of the prominent symptoms of the intolerance reaction.

Most of the intolerant individuals reacted to ingestion of ASA with symptoms of their basic disease (77.8%) (Table III). Practically all patients with chronic asthma, except two, reacted to ASA with an asthma attack. One patient with asthma exhibited angioedema, another showed a fixed exanthema on the right forearm after taking ASA. Patient I was seeking medical treatment for her little daughter who was suffering from asthma. Her intolerance to ASA was disclosed during investigation of the family history. She had no complaints other than this untoward reaction to ASA to which she reacted with generalized urticaria.

Seventeen of our 27 patients intolerant to ASA were skin tested with N-salicyloyl and N-acetylsalicyloyl PLL (antigens 1 and 2). No case showed a positive skin reaction of the immediate type. Thus it can be assumed that these patients lack skin-sensitizing homocytotropic antibodies (IgE) with N-salicyloyl or N-acetylsalicyloyl specificity. The presence of such antibodies were a prerequisite of the syndrome which these patients exhibited after intake of ASA were of allergic origin. To confirm these results sera of 17 patients were passively transferred to rhesus monkeys by intradermal injection of 0.05 ml of each serum. The antigen challenge

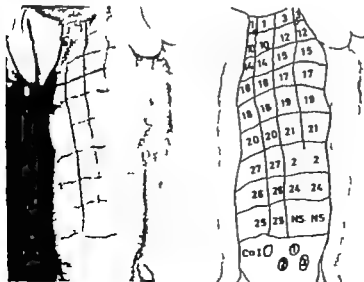


Fig. 1. Passive transfer of sera of ASA intolerant individuals into a rhesus monkey. The antigen challenge was carried out by intradermal injection of N-acetylsalicyloyl-PLL into the previously sensitized skin. The figures represent the patients' sera used. NS = normal human serum. Co 1 = antigen control 1-3 (bottom) = histamine control (10, 20 and 30 µg). The black spots within the marks indicate positive reactions. The additional marks at the bottom are additional marks at injection.

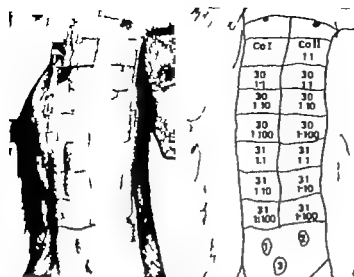


Fig. 2 Passive transfer of two sera from patients allergic to Timothy grass pollen (nos. 30 and 31). The antigen challenge was carried out by injection of Timothy grass pollen extract simultaneously with Evans blue C II = normal human serum. Other symbols as in Fig. 1.

was carried out either by i.v. (experiment I) or local (experiment II) application of two different antigen preparations (N-salicyloyl-BSA, N-acetylsalicyloyl-PLI) or by oral challenge with 50 mg ASAN or 500 mg ASA (experiment III).

The salicyloyl-specific conjugates were tested for their anaphylactogenic activity in guinea pigs sensitized with ASAN prior to their use in the monkey experiments. Intradermal application of histamine was used as a control in these experiments. None of the sera tested induced skin reactions after antigen challenge thus revealing the absence of homocytotropic antibodies specific for N-acetylsalicyloyl or N-salicyloyl determinants. Fig. 1 stands as an example for these experiments and shows the sensitized skin of monkey 30 min after antigen challenge with N-salicyloyl-BSA applied i.v. together with Evans blue. The skin-sensitizing properties of human homocytotropic antibodies in primates were demonstrated by application of the sera of patients allergic to Timothy grass pollen. The skin reaction induced by the injections of Timothy grass pollen extract together

with Evans blue 24 hours after intradermal sensitization with the sera is shown in Fig. 1.

The concentrations of the different immunoglobulins of ASA-intolerant individuals were found to be within the normal range (Table IV). This applies also to the concentration of IgE which, however, shows somewhat elevated levels in the ASA-intolerant group compared with normal individuals. In contrast to this, the IgE concentrations in the sera of 3 patients with an immediate type allergy to Timothy grass pollen has been found to be significantly elevated and ranged between 5 600 and 11 000 ng/ml.

The presence of immunogenic ASAN in commercial ASA preparations explains the occurrence of circulating antibodies with N-acetylsalicyloyl specificity in the serum of ASA-intolerant individuals and controls previously treated with ASA (11, 24, 57). These antibodies belong to the IgG class and are heterocytotropic (3) and able to sensitize guinea pigs passively (12, 57). We agree with the conclusion of Amos et al. (1) according to which these antibodies are not involved in an hypersensitivity mechanism.

Table III Immunoglobulin concentration in sera of 19 ASA-intolerant and normal individual (mean \pm S.D.)

	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	IgD (mg/100 ml)	IgE (ng/ml)
Normal range	958–1 613 (26)	152–342 (26)	40–220 (26)	0–3–30 (49)	60–1 000 (29)
Normal individuals	1 039 \pm 214	114 \pm 46	233 \pm 66	0.11 \pm 0.04	186 \pm 370
ASA-intolerant patients	1 166 \pm 281	197 \pm 82	146 \pm 55	0.31 \pm 1	344 \pm 904

DISCUSSION

The majority of patients exhibiting adverse reactions to ingestion of ASA described in the literature suffer from chronic asthma (16, 17, 20, 24, 27, 39, 43, 44, 56). This is in agreement with our findings, so is the fact that ASA intolerance occurs more frequently in females and appears to be manifested preferentially in middle aged patients. However, Falliers (15) has recently reported 25 cases of ASA intolerance in asthmatic children, chiefly in children with late onset of asthma (age range 9–15 y) and high incidence of nasal polyps. In contrast to many reports (Table II) only about 7% of our patients exhibited nasal polyps. According to the experience of one of us (P. K.) patients with chronic asthma frequently exhibit nasal polyps and the incidence of nasal polyposis seems not to be more frequent in ASA intolerant patients.

The majority of the ASA-intolerant asthmatic patients react to ingestion of ASA with an asthma attack which is sometimes combined with urticaria and/or angioedema (Tables I and III). One of our asthmatic patients reacted with a fixed exanthema to ASA and another with angioedema only. Vomiting occurred in 85% of our cases after intake of the offending drug. Although omitting has been reported only rarely (28, 45) and Pearson (43) denies explicitly that abdominal symptoms (pain or vomiting) are due to an intolerance reaction, it appears to us that this symptom is a part of the intolerance reaction, seems not to be related to the basic disease or to irritation of the stomach by the drug itself.

One of our patients tested with N-salicyloyl-PLL and N- α -ethylsalicyloyl/salicyloyl PLL exhibited immediate type skin reactions, neither did the passive transfer of sera of ASA-intolerant patients to rhesus monkeys result in positive skin reactions subsequent to intradermal challenge with N-salicyloyl-BSA and N-salicyloyl PLL or by oral challenge with ASAN and ASA, thus indicating the absence of reaginic antibodies in the skin and in the serum of the ASA-intolerant patients. The skin-sensitizing properties of human reaginic antibodies in monkeys are well established by others (34, 35) and have been demonstrated by us in control experiments with sera of patients allergic to Timothy grass pollen.

Thus our results show the absence of homocytotropic antibodies with N-salicyloyl or N- α -ethylsalicyloyl specificity in the skin and serum of individuals intolerant to ASA. Consequently we

must consider that the signs and symptoms occurring after intake of ASA (and in many cases after chemically unrelated drugs) are not of allergic origin but are due to another pathogenetic mechanism. Kallós (31) ascribed this syndrome already in 1956 to direct, not antibody mediated, histamine release through the offending drug, due to some inborn biochemical abnormality of intolerant individuals.

The biochemical mechanisms of the drug-mediated intolerance reactions are not known at this time, but a variety of hypotheses have been presented. Samter and Beers (46, 47) discuss a paradoxical reaction of kinin chemoreceptors to ASA and other analgesics resulting in stimulation instead of inhibition of the receptors causing bronchoconstriction and vasodilatation. Generation of anaphylatoxins by direct activation of complement components acting subsequently on release mechanisms of histamine or hypothetical deficiencies of enzyme inhibitors and activation of tissue enzymes by ASA, leading to an imbalance of enzyme and enzyme inhibitors in tissues followed by tissue damage and release of pharmacologically active mediators has been suggested (59). Interactions of analgesic drugs and prostaglandins are also to be considered in the pathogenesis of ASA-induced asthma (42). Prostaglandins of E series have been proved to be bronchodilatory, those of F series are usually bronchoconstrictive (8, 50). It has been shown too that ASA and other analgesics inhibit the synthesis of prostaglandins E and F in guinea pig lung (55).

Capplen and Haynes (7) described an abnormal factor in the serum and saliva of ASA-intolerant patients which disorganizes and inhibits the rhythmic beat of gill cilia of freshwater mussels and oysters *in vitro* within 10–30 min. This factor is regularly present in patients with cystic fibrosis of the pancreas and in less active form in their heterozygous parents. The authors discuss on this basis a possible genetic link between ASA intolerance and cystic fibrosis of the pancreas. The significance of these results is, however, doubtful. Wood and Di Sant-Agnese (58) have recently conducted an objective and thorough evaluation of the reliability of the assay of the circulating cilio-toxic factor in patients with cystic fibrosis of the pancreas and normal controls. They conclude that the "bioassays are highly subjective and completely unreliable in their present form". Obviously these assays have as yet no place among the accepted screening methods for cystic fibrosis (6).

Thus the discussion about the possible biochemical mechanism of ASA intolerance remains open. Further investigations are necessary.

In the last two years several reports on ASA intolerance in siblings have been published (7, 37, 41, 52). This suggests that genetic factors might be the ultimate cause of the intolerance. Strong evidence supporting this view has been provided recently in an ethnic isolate (Mennonites). Lockey et al. (37) found a family with high consanguinity. Four members of this family (3 first cousins) had asthma, nasal polyps and pronounced ASA intolerance. The authors stress that the presence of ASA intolerance "in these constellations of relatives suggests that it is inherited as an autosomal recessive trait". Were this the case, homozygotes would exhibit pronounced intolerance, whilst heterozygotes, e.g. the parents of intolerant individuals, would be free from obvious signs of intolerance and their state could eventually be disclosed by subtle methods such as challenge with ASA under controlled conditions. The usually late onset of ASA intolerance suggests the possibility that external factors play a role in its manifestation.

McDonald et al. (39) used oral challenge with ASA (0.640 g) in 42 selected patients with chronic bronchial asthma and nasal polyps who were not aware of any drug intolerance, especially intolerance to ASA. The challenge has been performed when physical examination and spirometry confirmed the absence of asthmatic symptoms. The patients received their usual maintenance medications, including cortisone, which usually prevented the occurrence of manifest asthmatic symptoms. After challenge they have been closely observed and spirometric values have been recorded at regular intervals. A rapid reduction of 50% or more of the FEV₁ was indicated a positive response. In this way ASA intolerance could be proved in 8 of the patients. They did not react to placebo tablets, other analgesics or tartrazine. With regard to the findings of Lockey et al. (37) these individuals are probably heterozygotes with partial phenotypic expression of their hereditary trait. Another example of this has been provided by Delaney (11) who described a family in which a 15-year-old girl presented with severe asthma, nasal polyps and ASA intolerance. Her father had chronic asthma but denied any drug intolerance. Oral challenge with ASA, however, elicited an asthmatic attack. It is tempting to assume

that the father is heterozygote, his daughter homozygote. Further investigations along these lines are necessary.

As mentioned above, about 70% of our ASA intolerant cases reacted with identical symptoms to a variety of structurally unrelated compounds too. This is in accordance with the observations of several other investigators (16, 17, 20, 22, 25, 29, 43, 44, 46, 51, 56). Samter and Beers (46) for instance reported 18 cases of ASA-intolerant asthmatics who reacted with asthmatic symptoms to therapeutic doses of indomethacin. Previous contact with the drug could be excluded. Juhnlin et al. (30) performed oral challenge in 7 ASA-intolerant individuals with ASA and several other drugs and food additives. The patients were aware of their ASA intolerance only. A food additive, tartrazine (FD & C yellow No. 6) in small doses (1-5 mg) elicited severe reactions (urticaria, angioedema and/or asthma) in all 7 cases. Derivatives of benzoic acid elicited similar symptoms in some cases. It is perhaps worthwhile to note that ASA free from its anhydride elicited severe reactions in all three cases challenged with it (doses 3.5-50 mg). It must be assumed that the pathomechanism of the reactions of ASA-intolerant individuals to structurally unrelated compounds is identical with that operative after ingestion of ASA. A number of cases react to antibiotics, mainly penicillin, too. These reactions are probably allergic. The signs and symptoms of drug intolerance identical with those occurring anaphylaxis, i.e. a more or less violent allergic reaction of the immediate type, cannot be regarded as sufficient criterion to implicate an allergic origin. Our results and the literature suggest, the syndrome is the sequelae of direct liberation of histamine (and other mediators) by ASA and other drugs. It should be properly denoted as "drug-induced anaphylactoid syndrome".

The ability of a variety of drugs to release histamine in certain individuals (thus inducing the anaphylactoid syndrome) is in our opinion due to a hereditary trait. During the past few years experimental and clinical studies have revealed correlation between histocompatibility antigens (HLA and LA in human) and the susceptibility to certain diseases. An altered frequency of the occurrence of one or more of these antigens in a group of individuals suffering from a disease strongly supports the possibility that the susceptibility is due to genetic factors in the host (2, 4, 5, 8, 10, 14, 1, 38).

We have started investigations in an attempt to determine whether in the subpopulation of asthmatics showing anaphylactoid reactions after ingestion of ASA and certain other drugs such an alteration of the frequency of HL A or LA antigens can be revealed. Recently La Du Jr (32) stressed that untoward reactions to drugs may be due to genetically determined factors such as the absence or deficiency of particular enzymes or the abnormality of a receptor. Such an "inborn error of metabolism" may be entirely innocuous for the individuals unless a particular drug is prescribed. It is an important aim for future research to find the inborn error of metabolism underlying the drug-induced anaphylactoid syndrome.

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VARIATIONS IN L-DOPA ABSORPTION

Report of a Parkinsonian Patient with High Plasma Dopa Concentration after Therapeutical L-dopa Dose

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Abstract In parkinsonian patient with weak effect of L-dopa on the parkinsonian symptoms, unusually high plasma levels of dopa and dopamine sulphate were noticed. The plasma concentration of tyrosine and phenylalanine were low during the whole observation period. The urinary excretion of dopamine sulphate and the ratio of dopamine sulphate to dopamine in urine were high. The reason for this high ratio of dopamine sulphate to dopamine could not be determined. The disappearance of L-dopa from plasma after administration was only slightly reduced. It was concluded that the L-dopa absorption in this patient was very efficient due to low dopa decarboxylation in the intestinal organs in spite of normal decarboxylase activity in other peripheral tissues. Such selective decrease in the dopa decarboxylation in the intestinal organs is presumably unusual, but illustrates that very marked individual variations in the L-dopa metabolism may occur in parkinsonian patients.

The therapeutical dose of L-dopa varies markedly in patients with Parkinson's disease. Our patient material between 1 and 8 g/day (3) and is not directly related to age, body weight, symptoms or duration of the disease (70). The plasma level of dopa varies considerably after the same oral dose in different patients (1, 7, 8, 11, 12, 14, 1, 24, 26, 77, 28). In relation to the administered dose the plasma level of dopa has been found to be low due to the fact that L-dopa is to a large extent decarboxylated already in the intestinal organs (1, 5, 10, 18). Furthermore the turnover rate of absorbed L-dopa is rapid (18).

We have, however, found one patient who had a very high plasma level of dopa on ordinary therapeutical dose. The studies performed in this patient indicated a low rate of dopa decarboxylation

in the gastrointestinal organs but a normal decarboxylation in other peripheral tissues including the kidneys.

CASE REPORT

The patient was a woman aged 76 years at the time of this study. At the age of 57 she had had symptoms of encephalomyelitis. Three years later the first symptoms of Parkinson's disease appeared. When the parkinsonian symptoms had persisted for five years thorough neurological investigation was made which confirmed the diagnosis. On this occasion the cerebrospinal fluid was examined and EEG and pneumoencephalography were performed with normal findings. Anticholinergic therapy was then started with certain effect at the beginning of the treatment but later the parkinsonian symptoms continuously increased. In 1969 the patient had a very advanced physical handicap and she needed assistance in most of the activities of daily living (ADL group 4) (20).

In 1969, before the start of L-dopa treatment, the cerebrospinal fluid was examined. The homovanillic acid (HVA) concentration was 6.0 µg/l which is a value similar to that found in control material. The value was higher than in most parkinsonian patients (19).

During the time of the study the patient had no signs of cardiac insufficiency and X-ray of the heart and lungs was normal. During the time of the L-dopa treatment (18 months) her body weight varied between 90 and 52 kg. She did not have any history of renal disease but the serum creatinine was 1.5 mg/100 ml (reference values 0.6-1.2) and the glomerular filtration 41 ml/min as determined by the method of Åberg et al (7). Urinary tests and routine haematological tests as well as liver function tests were normal.

L-dopa treatment was started in 1969 when the patient was 74 years old. At the maximal tolerable L-dopa dose of 3.0 g/day small effect on hypokinesia and rigidity could be seen, but the patient still belonged

one. In our patient not only the excretion of dopamine sulphate but also the ratio conjugated to non-conjugated dopamine was extremely high. This is a remarkable observation since after i.v. administration of L-dopa only about 6% seems to be excreted as dopamine sulphate whereas as much as 17% is excreted in free form (15) resulting in a ratio of about 0.4 between dopamine sulphate and free dopamine in urine. The ratio of conjugated to non-conjugated dopamine could therefore be expected to be low in a patient with an efficient L-dopa absorption and not high as observed in the present case. We do not know the reason for this high degree of dopamine conjugation in our patient.

The total excretion of dopamine and dopamine sulphate in urine was also large in relation to the oral L-dopa dose given. This shows that L-dopa was readily decarboxylated once it had reached the general circulation. It might also indicate that the further degradation to HVA which is the main metabolite was depressed. Unfortunately determination of the HVA excretion was not possible because the urine samples available contained salicylate which interfered with the estimation.

The somewhat lower elimination rate of i.v. administered L-dopa in this patient compared to the parkinsonian patients is in agreement with the assumption that there was a preferential inhibition of the dopa decarboxylation in the intestinal organs but a normal decarboxylation in other organs such as brain and kidney. The reason for such a selective inhibition is not known. A marked inhibition of the decarboxylase activity in the liver of rats has been observed (13) after chronic administration of L-dopa. However we have recently studied the degree of dopa decarboxylation in patients treated with L-dopa during 6-24 months without being able to demonstrate a reduced rate of dopa decarboxylation in the gastrointestinal organs (18).

L-dopa treatment has been reported not to influence the levels of the other plasma amino acids (1, 22). In the present patient the levels of tyrosine and phenylalanine however were constantly low. This might only partly be the result of a low protein intake as the levels of the other amino acids were normal. The low tyrosine and phenylalanine concentrations might also be an effect of the unusually high plasma dopa level

influencing the homeostasis mechanisms or as an effect of the high intestinal L-dopa absorption, resulting in a competitive inhibition of the absorption of structurally related amino acids. There appeared not to be a primary defect in the absorption of free phenylalanine as judged from loadings with this amino acid.

The conclusion is that in this case the majority of orally administered L-dopa was decarboxylated in the kidneys and not as previously demonstrated in other patients mainly in the gastrointestinal tract. The reason for the high dopamine sulphate production in our patient could not be determined. To what extent this anomaly in the dopa metabolism has influenced her clinical condition and the therapeutical response is not known. The present study shows that a conventional treatment of a patient with a classical Parkinson's syndrome can result in an extremely high dopa concentration in plasma and that marked variations in the L-dopa metabolism may occur.

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LACTIC ACIDOSIS IN EPINEPHRINE POISONING

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Abstract Lactic acidosis as result of epinephrine poisoning is discussed. A case report illustrates the clinical condition after very large dosage of epinephrine (20 mg i. v.). The pathogenesis of lactic acidosis in this situation is discussed and suggested to be of type II A and II B according to Huckabee. We advocate the treatment with insulin, glucose and bicarbonate.

Lactic acidosis was first mentioned in 1935 in dehydrated children (?) but this work did not provoke any investigation of the etiology of lactic acidosis until Huckabee (5) in 1961 analysed the causes of elevated lactate concentrations in the blood. Recently Oliva (8) has published a review on the subject. We have observed an unusual cause of lactic acidosis—epinephrine poisoning—and we here discuss the possible mechanism of lactic acidosis on the basis of the present case.

CASE REPORT

A 23-year-old male known to be morphine addict had injected 1 ml Hectalin = 70 mg epinephrine (verified by the police and the patient). In an attempt at suicide 11 hours prior to arrival in the Intensive Care Unit. Initially he was comatose with widely dilated pupils non-reactive to light, enormously constricted peripherally with cyanosis. Respiration was accentuated—Kussmaul-like—and tachycardia (120 beats/min) was present. BP 125/75 mmHg. ECG showed sinus tachycardia and isochronic changes in all leads. Gradually he became comatose and blood chemistry showed a heavy metabolic acidosis (Fig. 1). Ketonaemia was absent judged by the Acetest. BUN was normal.

Treatment was started with 3 g/kg administered through an endotracheal tube, 200 mEq bicarbonate in 4 hours, 70 IU crystalline insulin and 1700 ml isotonic glucose. Potassium supplement 30 mEq was given. Four hours after arrival the patient developed pulmonary edema and hypotension. After successful treatment with 0.30 mg ouabain, 100 ml 20% human albumin and 80 mg furosemide, his condition rapidly improved. Acidosis, anoxia and electrolytes became normalized.

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7 hours after arrival (Table 1). The patient, as discussed fully, was further supported treatment in the Psychiatric Department.

Laboratory data

Hb 1.9 g/100 ml hemoglobin 39.0% pO₂ 75 mmHg, arterial pH 7.07, p_aCO₂ 33 mmHg, Lundberg bicarbonate 9.2 mEq/l, serum sodium 135 mEq/l, serum potassium 3.6 mEq/l, BUN 14 mg/100 ml, serum bilirubin 0.9 mg/100 ml, prothrombin time 13.5, SGOT 65 U/l, normal value <40, plasma glucose 379 mg/100 ml, serum lactate 9.5 mEq/l (normal value 0.55-1.75), serum methanol 0. No acetone, blood glucose or albumin in the urine.

ECG showed sinus tachycardia with ischemic changes in all leads and X-ray thorax revealed pulmonary edema.

DISCUSSION

Lactic acidosis is metabolic acidosis characterized by a significant elevation of lactate concentration in the blood (4, 8). Clinically the condition is dominated by Kussmaul respiration and development of shock and coma (1, 8, 9, 11).

Huckabee (5) was the first to suggest classification of lactic acidosis into two main types. Type I is characterized by an elevation of both lactate and pyruvic acid so that the lactate:pyruvate ratio is constantly 10:1. In this type there is no excess lactate. In type II the lactate concentration is augmented without corresponding elevation of pyruvic acid; the lactate:pyruvate ratio will be increased and excess lactate will be present. This is often seen when hypoxia and/or circulatory failure dominate the clinical picture. This type has been subclassified as II A, in which hypoxia and desaturation of arterial blood are present, and as II B, in which no obvious signs of hypoxia are present. The last mentioned condition is often secondary to diseases with disturbed lactate elimination, renal and hepatic insufficiency.

Greene (3) found lactate levels of 12-9 mEq/l following epinephrine injections of 3 µg/kg in 70 min.

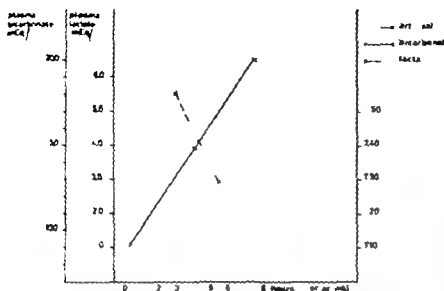


Fig. 1 Plasma lactate (—) plasma bicarbonate (---) and arterial pH (· · ·) during treatment with glucose, insulin and bicarbonate

in healthy human volunteers. We have found no investigation of the effect of very large doses of i.v. epinephrine on human subjects. Primarily epinephrine will stimulate the glycogenolysis resulting in augmented glycolysis (10). This will result in an increased lactate-pyruvate concentration, but the lactate-pyruvate ratio will be constant 10:1 (1). If large doses are given, the peripheral action of epinephrine will dominate, resulting in tissue hypoxia and lactic acidosis of type II will be produced. The decreased perfusion of liver and kidney (6) will further accentuate the acidosis, as the lactate elimination is greatly diminished. We suggest that the lactic acidosis in the present case was a combination of types II A and II B.

We treated the patient with insulin, glucose and bicarbonate (7). The only complication seen was acute forward failure, probably caused by the u-

chemic strain on the heart. As epinephrine is rapidly inactivated from the body, the treatment of choice in poisoning resulting in lactic acidosis will generally be supportive in regard to respiration and circulation. We do not find treatment with peritoneal or hemodialysis indicated in cases of lactic acidosis when the precipitating factor is known and no longer present.

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Table 1 Clinical signs observed in the patient during the first 7 hours after admission

++present --absent

	Hours						
	0-1	1-2	3	3-4	4-5	5-6	6-7
Polypnea	+		+		+		-
Coma	-	+		+	+	-	-
Peripheral constriction	+				(+)	-	-
ECG ischemia	+					-	-
BP	→	→	↓	↓	↓	→	→
Pulmonary edema	-	-	-	-	-	+	-
Acidosis	+++		+			-	-

FATAL POISONING WITH DEXTROPROPOXYPHENE-CONTAINING ANALGETICS-SUICIDE OR NOT?

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Abstract Among 63 cases of fatal poisoning with analgetics containing dextropropoxyphene alone or in combination with ethyl alcohol and/or other drugs suicide could be proved in only 20 cases. In a consecutive group of 63 cases of fatal poisoning with barbiturates 42 were regarded as suicide. Thus the number of other deaths turned out to be much higher in the dextropropoxyphene series. The uncharacteristic clinical course and the absence of specific pathomorphological findings at autopsy mean that such intoxications can easily be overlooked.

Analgetics containing dextropropoxyphene were introduced in the middle of the 1950s and have since been widely used and prescribed not only by physicians but also by dentists. In Sweden pure dextropropoxyphene chloride is a component of the compounds Dolotard and Doloxene®. It is combined with salicylic acid, caffeine and phenacetin in capsules (Doloxene comp N and comp forte N). T. Doleron® contains also trantergan (2-diethylaminoethyl-phenothiazine-carboxylate chloride) and phenacetin has been replaced by phenazone. T. Doleron matt® contains not only the substances in Doleron® but also a barbiturate, vinylbarbital (5-ethyl 1-methyl-butenyl-barbiturate) instead of caffeine. Other dextropropoxyphene preparations are combined with salicylic amide (T. Dolomud forte®), chlorzoxazone + salicylic acid (T. Paraflex comp N®), paracetamol (T. Distalgic®), phenaglycodol + salicylic acid (Caps. Vondar®), phenprobamate + salicylic acid (T. Tergiflex®) and in the latter preparation also pentobarbital (T. Tergnox®).

Recent years have witnessed an increase in the frequency of fatal poisoning with such analgetics often combined with ethyl alcohol or barbiturates.

In a strikingly large percentage of these cases it could not be proved that overdosage occurred with suicidal intent, such as is often the case in fatal poisoning with soporifics. To further evaluate this impression a consecutive series of 63 such cases of fatal poisoning with dextropropoxyphene-containing analgetics was studied regarding the mode of death and compared with an equal number of cases of poisoning with barbituric acid.

MATERIAL AND METHODS

Analgetic containing dextropropoxyphene (series I)

The material consisted of 63 cases examined post mortem between Feb. 1971 and Jan. 1974. The age and sex distribution is given in Fig. 1. Three of them were cases of fatal poisoning with Doleron (dextropropoxyphene chloride, phenazone, caffeine, acetylsalicylic acid, trantergan) alone and 60 with combinations of analgetics containing dextropropoxyphene and barbiturates (e.g. T. Doleron matt®) or alcohol or psychopharmacological drugs.

Only cases fulfilling one or more of the following criteria were regarded as suicide: 1) Discovery of suicidal note written by the deceased. 2) Pronounced aversion to living or known depression. 3) Earlier attempted suicide. 4) Examination revealing large amounts of the toxic substances in various organs. Cases in which it could not be decided whether death had been suicidal or not, the relatives were contacted later. No new information confirming suspected suicide was ever obtained.

The chemical analyses were performed in the Government Laboratory for Forensic Chemistry, Stockholm. The following method was used. The liver tissue was homogenized and extracted with ethyl alcohol. The extract containing about 70% ethyl alcohol. Extract of ethyl alcohol in an amount corresponding to 10 g liver tissue was purified according to the cooling method (1).

Dextropropoxyphene and other substances, for example salicylic acid, phenazone and barbiturate derivatives,

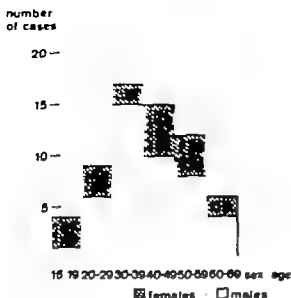


Fig 1 Age and sex distribution of the dextropropoxyphene material.

were extracted with chloroform from weak acid solution (pH -3) in the form of the soluble hydrochloride. The chloroform extract was analysed for acid compound (UV spectrophotometry paper chromatography), and neutral compound (UV spectrophotometry thin-layer chromatography and colour reaction). Dextropropoxyphene analyses were mainly performed by gas chromatography. The glass columns were filled with one of the polymers SE 30, Hi-Elf and OV 17 as stationary phases and placed on carrier material of chromosorb and/or gaschrome-Q-type whereafter the substance eluted could be measured. The identity of the dextropropoxyphene peak was crafted by mass spectrometry. For quantitative calculations the gas chromatography peak in liver extract corresponding to 4 mg liver was com-

pared with the peak of 0.1 µg dextropropoxyphene hydrochloride standard substance (2).

Barbituric acid poisoning (series II)

The consecutive series comprised also of 63 cases analysed in the same fashion as in series I. In these cases the relatives were not contacted anew. The analysis included examination for ethyl alcohol, barbiturates, meprobamate, metaxalone, benzodiazepine derivatives, hydroxyzine, hexapropylamine, phenothiazine derivatives and tricyclic amines.

RESULTS

Analgesics containing dextropropoxyphene

In only 70 of the 63 cases had investigation by the police given reason to suspect intentional poisoning. In all the other cases the police had requested postmortem examination because death had been sudden and the cause obscure. In these cases the physicians were not prepared to issue a death certificate on the basis of the results of the investigation by the police, knowledge of prior illnesses of the deceased and/or external inspection of the corpse. Postmortem examination had not revealed any morphologically clear evidence of the cause of death in these latter cases either. Tablets containing dextropropoxyphene are often completely dissolved in the stomach for which reason the absence of fragments of such tablets does not exclude the possibility of poisoning by such substances. For this reason chemical analyses were carried out by the Government Laboratory for Forensic Chemistry.

Table 1 Patient data of 4 cases in series I, estimated drug consumption and concentrations of substances in liver tissue and ethyl alcohol in the blood

BARB=vinbarbital, SAL=salicylic acid, PHEN=phenazone, DPO=dextropropoxyphene, ETH=ethyl alcohol

Sex	Age (y)	Interval between last seen living and found dead	Drug consumption according to police investigation	Concentrations of substances in liver tissue (mg/100 ml) and ETH in blood (%)				
				BARB	SAL	PHEN	DPO	ETH
♂	32	3 d.	2 Doleron + 23 Doleron matt®	1.6	1.6	0.7	0.7	-
♀	42	1 d.	11 Doleron matt + 9 Lunedon®	1.3	5.6	6.7	1.8	-
♂	36	2 h	30 Doleron matt	1.4	1.5	6.2	3.0	1
♂	32	3 h	1. Doleron matt	0.9	1.6	3.0	.0	1.4

Table II. Division into groups according to substances found in liver tissue and ethyl alcohol in blood and number of suicides or other deaths (accidental, intentional or natural)

Group	Suicides	Other deaths
1 DPO-SAL-PHEN	3	0
2 DPO-SAL-PHEN-BARB	6	6
3 DPO-SAL-PHEN + ETH in blood	2	1
4 DPO-SAL-PHEN-BARN + ETH in blood	2	18
5 Different combinations of DPO-SAL-PHEN + ETH + some of the following: isopropanol, benzodiazepines, barbiturates, methaqualone, chlorzoxazone, lacticopropylamine and tricyclic amines	7 20	7 43

Abbreviations as in Table I.

The chemical analyses were generally performed on liver tissue. The concentrations of the substances found varied considerably: phenazone 1-15.1 mg/100 g, salicylic acid 0.5-35 mg/100 g, dextropropoxyphene 0.2-28.7 mg/100 g and barbiturates of vinbarbital type 0.28-7.3 mg/100 g. A Doloron[®] tablet contains 65 mg dextropropoxyphene chloride, 350 mg acetylsalicylic acid, 150 mg phenazone, 50 mg caffeine and 5 mg trantergan. (No method is available for demonstrating trantergan.) T Doloron mini[®] also contains 50 mg vinbarbital instead of caffeine. No certain relation was found between the amount of drug taken when known and the tissue concentration of the substance by chemical analysis. This may to certain extent be due to different durations of the periods between death and post mortem examination, and it could therefore not be determined how much of the substances had been excreted or metabolized (Table I). Neither was the duration of survival after ingestion of the drug known. It is also remarkable that no correlation was found between the concentration of the substance in the liver tissue and the relative amounts of the substances in the tablet.

The material was divided into five groups according to the substances found by chemical analysis (Table II).

Of the total number of cases, the dextropropoxyphene series 20 (about 3%) were classified as suicide. In 13 of these cases it was clear

from the preliminary police reports that poisoning had been suspected.

In group 1 (age range 19-20 years) i.e. cases of poisoning with pure Doloron[®], all deaths were judged to be suicides and witnesses had seen one patient take a handful of Doloron[®] tablets. The concentrations of the components in the liver were as follows: dextropropoxyphene 3.8-28.7 mg/100 g, salicylic acid 3.8-35 mg/100 g and phenazone 6.0-15.1 mg/100 g.

As regards group 2, according to the investigation by the police, a varying number of different packets of tablets containing soporifics, psychopharmacological drugs and analgetics were found at discovery of the deceased. But containers of T Doloron mini[®] were found only in 5 of the 18 cases. In this group the concentrations of the substances in the liver varied as follows: vinbarbital from 1.1 to 3.7 mg/100 g, salicylic acid 1.4-9.8 mg/100 g, phenazone 1.9-13.0 mg/100 g and dextropropoxyphene 0.2-1.0 mg/100 g.

For group 3, analgetics containing dextropropoxyphene + ethyl alcohol, concentrations of the components in the liver and of ethyl alcohol in the blood are given in Table III.

One of the cases in this group was remarkable. While under the influence of alcohol a 77-year-old chronic alcoholic (the last case in Table III) had taken 15 Dolotard capsules. One capsule contains 0.15 g dextropropoxyphene chloride. He died

Table III. Liver weights and concentrations of substances in liver tissue in group 3

Sex	Age (y)	Liver wt. (g)	Concentrations of substances in liver tissue (mg/100 ml) and ETH in blood (‰)			
			DPO	SAL	PEN	ETH
♂	36	190	6.3	5.0	3.3	1.7
♂	41	1920	0.5	0.1	1.1	9
♀	46	1670	6.0		5.6	1.0
♂	35	1510	1.7	7	0	1.9
♂	61	1800	11	8	4.9	1.9
♂	53			0.9	8.0	1.7
♂	47	1710	0.3	1	1.9	1.3
♂	43	1400	0.6	7	3.7	1.4
♂	57	1690	1.8		1.3	1
♂	4	2140	1	10	1	0.6
♂	61	1790	0.4	15	14	4
♀	16	1430	7.0	4.0	11	0.6
♂	28	30	1.9	6	1.0	1.9
♂	77	830				0.9

Abbreviation as in Table I.

six hours later. Chemical analysis by the Government Laboratory for Forensic Chemistry revealed only dextropropoxyphene and ethyl alcohol. The autopsy revealed no pathological changes in the internal organs beside pulmonary oedema. As far as is known, this is the first case of fatal poisoning with pure dextropropoxyphene chloride and ethyl alcohol on record.

Regarding group 4, analgetics containing dextropropoxyphene and barbiturate of vinbarbital type + ethyl alcohol, packets of Doleron natt® tablets were found in 9 cases. In this group the concentrations of the substances in the liver were as follows: dextropropoxyphene 0.2–17.7, salicylic acid 1.1–11.3, phenazone 1.3–9.1 and barbiturate of vinbarbital type 0.28–7.3 mg/100 g. The concentration of ethyl alcohol in the blood ranged between 0.3 and 2.4 ‰.

Pulmonary oedema was revealed in all cases at autopsy. No specific pathoanatomical changes were demonstrable. Fragments of the tablets were found in the stomach in 79 cases, including 15 with the above mentioned combined poisoning with other tablets.

Barbituric acid poisoning

In this series 42 (about 67%) of altogether 63 cases were judged to be suicidal. Combined intoxication with ethyl alcohol was noted in 32 cases. In 11 of the cases of suicide and in 14 of the obscure cases the fatal poisoning was also accompanied by ethyl alcohol.

DISCUSSION

Drugs containing dextropropoxyphene are available in Sweden as described in the introduction. Inquiry among the pharmaceutical manufacturers revealed that in Sweden (about 8 mill. inhabitants) at least 90 mill. tablets of these preparations were sold in 1973. This illustrates the present extensive use of drugs containing dextropropoxyphene. The risk of poisoning is therefore considerable. For comparison it might be mentioned that the corresponding figure for soporific tablets containing barbituric acids was at least 60 mill. The number of cases of fatal poisoning with these preparations examined post mortem in the Department of Forensic Medicine, Lund, in 1973 was 101, the corresponding figure for cases of poisoning with

dextropropoxyphene-containing analgetics during the same period was 43.

The first case of fatal poisoning with pure propoxyphene hydrochloride was described in 1964 (6). Since then several cases of fatal poisoning with compounds containing propoxyphene and dextropropoxyphene have been reported (7, 8, 9, 10). At autopsy only pulmonary and possibly cerebral oedema was demonstrated. The clinical course has been described as deep coma, tendency to convulsions and inhibition of the respiratory and circulatory centers (5). One case has been reported with repeated respiratory arrest alternating with wakefulness (4).

Analysis of the above material showed that poisoning by drugs containing only dextropropoxyphene, salicylic acid, phenazone and trantergan is only rarely fatal. In all 3 cases in the present material such fatal poisoning was always regarded as suicide. In the remaining 60 cases the fatal issue was ascribed to a combination of the above mentioned substances with either barbiturates with or without ethyl alcohol or a combination of psychopharmacological drugs.

It cannot of course be excluded that additional substances not demonstrable by the analytical methods used had been taken by the deceased.

The question is whether any existing disease might have contributed to the fatal issue in the cases observed. In only two of the cases did autopsy, which was mostly supplemented by microscopic examination, reveal a serious disease which might have influenced the course of events. One of these was a 43-year-old male diabetic and the other a 56-year-old man with cardiac hypertrophy. In the former case autopsy showed mild myocardial fibrosis and moderate fatty degeneration of the liver. In the second case left ventricular hypertrophy with a heart weight of 550 g and bilateral renal cysts. In three additional cases autopsy revealed advanced arteriosclerotic changes in the coronaries and in one case diffuse myocardial fibrosis. No signs of recent ischaemic myocardial lesions were demonstrable in these cases at microscopic examination with Mallory-PTAH staining. In five further cases there was fatty degeneration of the liver, possibly owing to chronic abuse of ethyl alcohol.

Perusal of the police reports available and the hospital records did not reveal any cases in which analgetics containing dextropropoxyphene had

been prescribed because the patient was suffering from some disease which might have been of importance for the fatal outcome. In 36 cases the preparation had been prescribed for the deceased himself and in 6 for relatives or friends. In the other 1 cases it is not known how the deceased had come by the drugs.

The course of poisoning with dextropropoxyphene-containing analgetics combined with alcohol and/or barbiturates, psychopharmacological drugs, etc. is very uncharacteristic as is apparent from the following observations.

While eating a meal a 41-year-old man who had been drunk almost every day for some months, swallowed a handful of tablets in the presence of witnesses. He then fell asleep and four hours later he was found dead.

A 28-year-old man had taken an unknown number of tablets while under the influence of alcohol. He afterwards felt ill and ordered a taxi to a hospital. He had got out of the taxi but could not pay the fare. After consultation with the personnel at the hospital he returned home in the same taxi to fetch the money. He lost consciousness on the way home and died 1 hour later.

At about 8 p.m. a 32-year-old man known to have abused drugs and alcohol was taken into custody by the police because under the influence of alcohol. At the police station he was deprived of all his tablets. It was found that he had escaped from prison. Therefore, at 2 a.m. by which time he had sobered up, he was returned to the prison. All his possessions and clothes were taken from him and he was given prison clothes instead. At 8 a.m. in the morning he was found dead. This person had thus not had the opportunity to take any tablets after he had been caught.

It is remarkable that, after having taken dextropropoxyphene-containing analgetics together with alcohol, the patients need only show signs similar to mild alcoholic intoxication and they may suddenly lose consciousness and die soon thereafter. It is not known whether this delayed effect can be ascribed to transference.

Autopsy revealed remains of tablets in the stomach in 10 cases (including 15 with combined intoxication) but not in the remaining 34 cases, though death apparently occurred relatively soon after ingestion of the tablets. The lack of anatomical changes of poisoning and the uncharacteristic course of poisoning clearly show how easily

such intoxication can go unnoticed during the police investigation and autopsy. This holds especially for cases with demonstrated pathological cardiac changes which could by themselves explain a sudden death.

It is also interesting to note that the inter-individual differences in the relation of the amounts of salicylic acid, phenazone, dextropropoxyphene and barbiturates in the liver bore no constant relation to the amount of ingested substances. This has been pointed out earlier by Boonichsen and Sköld (3).

As noted, only 70 of the 63 cases of fatal intoxication could be classified as suicidal. In the other cases the cause of death must be regarded as obscure. Whether the deaths in these obscure cases were accidental is difficult to determine. The tablets might nevertheless have been taken in an attempt to commit suicide. The deaths might also be explained by lack of respect for or knowledge of the effects of the drugs or lack of judgement while under the influence of alcohol. It is well known that alcoholics are prone to intensify the effect of alcohol by taking soporifics or psychopharmacological drugs. Moreover, the tablets may have been taken not to commit suicide but to attract attention or arouse pity by placing the deceased in a situation calling for help and relief in order to obtain a solution to personal problems. As for soporifics, the risk of overdosage is probably generally known also to those who combine the consumption of alcohol with soporifics. But when it comes to preparations described as "pain killing" muscle relaxing or night analgetics, one can hardly expect the laity to know anything about the risk of overdosage when combined with alcohol and/or soporifics. This may explain why fatal poisoning with dextropropoxyphene-containing analgetics together with alcohol or soporifics must be considered to be uncertain as regards suicidal intent or accident, in contrast to poisoning with barbiturates.

In view of the above mentioned varying concentrations of the degradation products of dextropropoxyphene-containing preparations in the tissues, caution must be exercised in the evaluation of suicide if only high concentrations of the substance are found in the organs of the deceased.

It is well known that consumption of ethyl alcohol can have synergistic effect on barbiturates. One should suspect such synergism in persons who

have consumed dextropropoxyphene-containing analgetics and ethyl alcohol or barbiturates. However, a search of the literature failed to reveal any information on this assumption.

The frequency of such poisoning with combined dextropropoxyphene-phenazone-salicylic acid and barbiturates (e.g. Doleron nati[®]) suggests that it is not wise to prescribe large quantities of such drugs at one time. This applies in particular to persons who might be thought to be alcoholics or to abuse barbiturates or who have mental problems.

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HAEMODYNAMIC EFFECTS OF DIHYDROERGOTAMINE IN PATIENTS WITH POSTURAL HYPOTENSION

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Abstract. In four patients with postural hypotension the effect of L- and oral administration of dihydroergotamine (DHE) has been studied. The therapeutic effect of oral administration of DHE has also been compared to the effect of treatment with an anti-gravity suit. Heart rate, intra-arterial BP, cardiac output (dye dilution technique) and central venous pressure were measured in supine position before and during simulated orthostatic stress induced by application of lower body negative pressure. In three patients forearm blood flow using venous occlusion plethysmography was also measured. Measurements were made before and after L- injection of DHE (0.01 mg/kg b. wt.). The long-term effects of oral administration of DHE are evaluated using an orthostatic test on tilt table before and during medication. Following L- administration of DHE, systemic and central venous pressures increased in all cases. The effect on cardiac output was negligible and consequently the calculated total peripheral vascular resistance increased slightly in all cases. Forearm blood flow changed in proportion to cardiac output. Orthostatic tolerance increased in all cases. Following long-term oral administration an increased orthostatic tolerance was demonstrated in three of four cases. The effect was comparable to that obtained with an anti-gravity suit. Thus acute and long-term administration of DHE can increase the tolerance to orthostatic stress in patients with severe postural hypotension. This is probably explained by effects on both capacitance and resistance vessels.

Postural hypotension is a rare disturbance of the reflex cardiovascular regulation of arterial blood pressure (BP) and heart rate (HR) (5, 6, 15). The most apparent and easily demonstrated manifestation of postural hypotension is a marked fall in arterial BP with no or at best only a slight increase in HR on head-up tilting. Though rare these patients present a great therapeutic problem (2, 11, 14). Concerning therapy three main

approaches are usually considered, measures aiming 1) an increase of the blood volume, 2) an increase of the peripheral vascular resistance, 3) a reduction of venous pooling in upright position. Thus mineral corticosteroids are supposed to operate mainly by increasing the blood volume while sympathicomimetic drugs increase the peripheral vascular resistance. Anti-gravity suits reduce pooling of blood in the lower body on transition from lying to upright position (16). Anti-gravity suits are often effective but their usefulness is often limited by the inconvenience experienced by these mostly elderly patients. However, modern modified anti-gravity suits may be a valuable complement to the therapeutic arsenal as shown by Rosenhamner and Thorslund (18).

In a recent study Nordenfält and Mefander (13) demonstrated that, in patient with so-called sympathicotonic orthostatic reaction, dihydroergotamine (DHE) exerts its effect mainly through constriction of the capacitance vessels thereby reducing venous pooling without demonstrable effect on the resistance vessels. Thus DHE could be expected to act as a pharmacological anti-gravity suit. The chemically related non-hydrated alkaloid ergotamine tartrate was reported to be of value in controlling postural hypotension as early as in 1946 by Berkenstam (3).

The above mentioned observations motivated the present study concerning the haemodynamic effects of DHE in patients with postural hypotension.

MATERIAL

Four patients, one male and three females, with postural hypotension were studied. Their age varied between 71 and 81 years. All patients presented the

Table 1 *Effect of head-up tilting on blood pressure and heart rate in four cases with postural hypotension*

Case no	Tilt angle	Systolic BP (mmHg)		HR (beats min ⁻¹)	
		Supine	Head-up tilted	Supine	Head-up tilted
1	90°	215	90	98	57
	80°	165	94	62	70
3	80°	105	Syncope	78	83
4	60°	155	85	90	87

typical picture of postural hypotension with prompt fall of arterial pressure and no or little increase in HR on head-up tilting. Systolic BP decreased to below 100 mmHg and HR increased by less than 10 beats/min in all cases (Table 1).

CASE REPORTS

Case 1

A 73-year-old woman. Since 2-3 years dizziness and occasional fainting in the erect position. During the last year unable to stand upright for more than a short time. Antigravity suit tried with partial relief. However the patient found the suit cumbersome and asked for alternative therapy. No signs of cardiac decompensation. ECG showed sinus rhythm and slight ST-T changes. There was slight general cardiac enlargement.

Case 2

An 81-year-old woman. Since many years attack of dizziness and fatigue in the upright position. Shortly before admission to hospital considerable deterioration. ECG still normal. ECG N cardiac enlargement and no signs of decompensation.

Case 3

A 73-year-old woman. Since some months attacks of severe dizziness and occasional epileptic seizures for which she received antiepileptic medication. Admitted to hospital because of these symptoms and difficulties in swallowing. Investigation including oesophagoscopy revealed non-malignant constriction of the cardia region. No signs of cardiac decompensation or cardiac enlargement. ECG showed sinus rhythm and ST-T depressions.

Case 4

A 71-year-old man. Typical symptoms of postural hypotension since 7 years. Initially good relief by the use of an antigravity suit. Deterioration since 3 years and during last year mostly bedridden. On physical examination no signs of cardiac decompensation. ECG showed atrial fibrillation and ST-T depressions. No cardiac enlargement.

METHODS AND EXPERIMENTAL PROCEDURE

Haemodynamic studies after 1 administration of DHE

The patients received no premedication. All drugs used for treatment of their postural symptoms had earlier been withdrawn so as not to interfere with this investigation.

Intrathoracic BP was measured through a short teflon catheter inserted percutaneously into either brachial artery. Central venous pressure was obtained by percutaneous introduction of a teflon catheter via an ante-cubital vein. The position of the catheter tip was checked by fluoroscopy and/or by respiratory pressure changes typical of intrathoracic recordings. Intravascular pressures were measured using strain gauge transducers (Statham P23Db) and recorded on an ultraviolet recorder (ABEM Ultratette). The reference level for pressure recordings was the mid-thoracic level in the supine position. Mean pressures were obtained by electrical integration. HR was obtained from the ECG. Cardiac output was determined by dye dilution technique using indocyanine green as indicator (Beckman Cardiolabometer). Forearm blood flow was measured using an air-filled plethysmograph according to Dohn, modified according to Graf and Westersten (10). A rough estimation of changes in venous tone was obtained by comparing the volume increment to a forearm segment caused by inflation of a collecting cuff to 30 mmHg above the pressure necessary to cause a barely detectable volume change of the segment (9).

Recordings were first made during control condition in supine position and then approximately 3 min after application of negative pressure around the lower body (LBNP). During the LBNP procedure the lower body (below iliac crest) was enclosed in an airtight box with the patient resting in horizontal position. The pressure within the box was reduced with an electric pump. The whole procedure was then repeated 10 min after administration of 0.01 mg DHE/kg b. w. The LBNP was adjusted in every case so as to ensure the BP and HR reactions during the initial tilt test.

Long-term effect of oral administration of DHE

After withdrawal of all treatment for postural symptoms, BP and HR were recorded at rest in supine position and after head-up tilting to an angle of 60-90°. The tilt angle had to be varied individually according to the severity of symptoms. BP was measured using a sphygmomanometer cuff. HR was determined from the ECG. This procedure was repeated after application of an antigravity suit inflated to pressure of 40 mmHg. The antigravity suit used was designed for medical use (Camp Scandinavia).

The patients were then reassessed with a similar tilt test 1-7 weeks after administration of a daily dose of 4-10 mg DHE. In cases 1 and 2 the blood volume using the carbon monoxide rebreathing method (17) was determined before and after approximately 1 month treatment. In order to avoid the effect of diurnal variation in orthostatic reaction all examinations

were performed at approximately the same hour of the day.

RESULTS

Circulatory effects of LBNP During LBNP systolic BP fell markedly in all cases—more than the diastolic pressure—and consequently the pulse pressure decreased (Fig. 1). In case 3 the BP was higher in the control situation than during the initial tilt test, presumably because the patient was somewhat dehydrated during the initial test.

Central venous pressure and cardiac output decreased in all cases during LBNP (Fig. 2). The decrease in cardiac output was mainly caused by a marked decrease in stroke volume (on an average 38%). The calculated total peripheral vascular resistance decreased in case 1 and was relatively unchanged in the other patients.

Forearm blood flow was measured in three cases. It was unchanged in case 4 and somewhat decreased in cases 1 and 3 (Fig. 2). Thus forearm blood flow did not show the marked decrease normally found during LBNP.

Venous tone measured as venous volume as described under Methods showed no detectable change during LBNP.

Effect of 1 administration of DHE During control condition DHE caused increases in systemic BP and central venous pressure in all cases (Fig. 3). Cardiac output was essentially unchanged after DHE and consequently the calculated total peripheral vascular resistance increased. There was no consistent change in forearm blood flow.

During LBNP arterial BP was maintained at a higher level following DHE (Fig. 2). In all cases cardiac output was somewhat larger during LBNP following administration of DHE. HR increased in all cases after administration of DHE. Stroke volume decreased much less during LBNP after administration of DHE (a reduction of 12% compared to 38%). The calculated total peripheral vascular resistance was higher in all cases. The central venous pressure fell to about the same level as before DHE.

Forearm blood flow showed only small and insignificant changes. Venous tone which increased after administration of DHE was not significantly changed during LBNP.

Side-effects Except for case 1 who experienced a transient sensation of pressure in the fore-

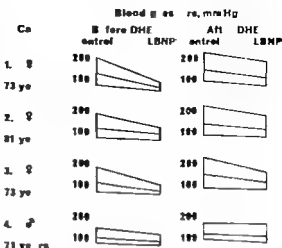


Fig. 1 Effect of lower body negative pressure (LBNP) on systemic BP before and after administration of 0.01 mg dihydroergotamine (DHE/kg b.w.t.m) in four cases with postural hypotension.

head subsiding within 8 min there were no subjective symptoms. No ECG changes were observed.

Effect of long-term oral administration of DHE The effect of DHE was compared with the effect of an inflated antigravity suit. Each patient was examined with a tilt test with and without inflation of an antigravity suit. After more than 3 weeks oral therapy with 4–10 mg DHE daily a new tilt test was performed (Fig. 3). Without treatment all patients experienced severe dizziness during tilting. With an antigravity suit inflated to 40 mmHg BP was maintained at a higher level during the tilt test and the above mentioned symptoms were partially relieved in all cases except no. 4. It may seem as if the difference with inflated antigravity suit, being no more than about 35 mmHg in two cases is relatively modest. However this was sufficient to accomplish marked relief of symptoms.

When the tilt test was repeated after oral administration of DHE arterial BP was maintained on the same average level as that obtained with an inflated antigravity suit. Patient 4 who had atrial fibrillation and a very low cardiac output, could not be improved by either inflation of an antigravity suit or administration of DHE orally.

Side-effects Patient 1 experienced slight gastrointestinal discomfort when receiving more than 4 mg DHE daily.

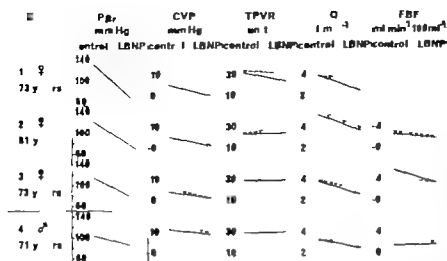


Fig 2 Effect of lower body negative pressure (LBNP) on some haemodynamic variables before (-) and after (---) administration of 0.01 mg dihydroergotamine (DHE)/kg b.wt. in four cases with postural hypotension.

DISCUSSION

In order to obtain a constant reference level for intravascular pressures LBNP was chosen instead of the usual tilt test to achieve a blood volume displacement (1, 8). This is especially important when measuring the low central venous pressure. In each case the LBNP was adjusted so as to obtain approximately the same BP and HR responses as had been obtained during the initial tilt test.

During LBNP cardiac output decreased while there was no change in forearm blood flow indicating a lack of reflex constriction of peripheral resistance vessels. Deficient reflex control of resistance vessels is probably the most important factor for the inability of these patients to maintain normal BP during orthostatic stimulus.

When analysing the effect of DHE on healthy normals and sympathicotonic orthostatic patients Nordenfält and Mellander (12, 13) have shown that DHE exerts a selective and powerful constrictive effect on capacitance vessels without much effect on resistance vessels. In accordance with these authors we have found that DHE increases systemic BP and central venous pressure.

This increase in central venous pressure was not accompanied by any change in heart volume after more than two months of oral therapy. In the study by Nordenfält and Mellander (13) on patients with sympathicotonic orthostatism the observed increase in cardiac output is sufficient to

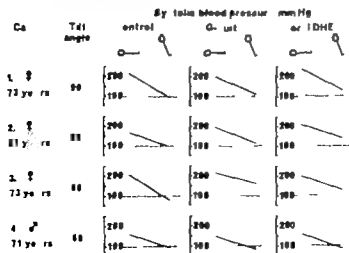


Fig 3 Effect of head-up tilting of systolic BP during control conditions after infusion of an antigravity suit and following more than three weeks of oral administration of dihydroergotamine (DHE).

explain the increase in BP. In the present cases with postural hypotension i.e. defective sympathetic vasomotor control cardiac output was essentially unchanged and thus the increase in BP must be explained by a constriction of resistance vessels. The explanation of this discrepancy considering the effect on the resistance vessels may be a difference in sympathetic nervous outflow to the vessels. Thus, during a blockade of sympathetic nerves as during spinal or epidural anaesthesia in man, a constrictive effect on the resistance vessels has been demonstrated (4-7). Recent studies indicate that the constrictive effect is limited to parts deprived of their sympathetic tone (7). This is in agreement with studies by Mellander and Nordenfält (11) who in cats observed a constrictive effect of DHE on resistance vessels only after sympathectomy.

However the demonstrated increase in peripheral venous tone after administration of DHE helps to reduce venous pooling and the decrease of cardiac output during LBNP and thus contributes to maintain BP on a higher level.

The present results show that administration of DHE to patients with postural hypotension increases systemic BP and orthostatic tolerance. In the present small material the effect is well comparable to treatment with an antigravity suit. However the increased orthostatic tolerance is obtained at the price of an increased systemic BP in recumbent position. This however probably applies also to most other forms of treatment.

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PRACTOLOL-PROPRANOLOL

A Comparison of Antihypertensive Effect

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Abstract. The antihypertensive effect of practolol and propranolol have been compared in a double-blind cross-over study on 4 patients with mild to moderate hypertension. Practolol gave the same BP reduction with less reduction in heart rate.

It has been demonstrated that many β -adrenergic blocking drugs have BP-lowering action. Propranolol (Inderal®) is most widely used and effective (8, 10, 11, 16). Propranolol appears to be a drug of comparable potency to betanidine, guanethidine and methyldopa (10). The hypotensive action of propranolol is not quite clear but oral administration induces a fall in BP associated with a reduction of cardiac output. Total peripheral resistance is initially increased (6, 14). Propranolol may provoke heart failure and bronchospasm in some patients. Increased peripheral resistance might explain clinically observed side-effects like Raynaud's phenomenon and cold hands and feet. Disturbances of sleep indicate central nervous action of the drug (10, 12, 16).

Practolol (Erakdina) is a β -adrenergic blocking drug which is relatively cardio-selective with much less effect than propranolol on the bronchial tree (9) or on peripheral vascular resistance (2, 3). Practolol does not show the quinidine-like effect of propranolol and affects cardiac output very little (2, 7). The drug does not pass the blood brain barrier (1).

Practolol seems to reduce arterial BP (5, 11, 15) without increasing peripheral vascular resistance. From a theoretical point of view according

ly the drug may be superior to propranolol in the treatment of hypertension with fewer expected side-effects.

The aim of this study was to compare the BP lowering effect of practolol and propranolol as single drugs on previously untreated patients with mild or moderate hypertension.

MATERIAL AND METHODS

Thirty previously untreated hypertensive patients of average age 53 years (range 18, 27 men and 3 women) entered the trial. All patients were recruited from present population study and had casual BP more than 175 and/or 115 mmHg on two consecutive readings. They were then followed until the BP stabilized after 3-6 out-patient controls. All had benign essential hypertension. Patients with history of bronchospasm, cardiac failure or intermittent claudication were excluded. The female patients did not use oral contraceptives. Six patients were excluded because of tablet failure.

All patients were examined and followed in the Hypertension Clinic Sahlgren Hospital. The examination included ECG and X-ray of the chest, urinalysis of albumin and glucose, serum creatinine, urea acid and electrolytes. Eye-ground examination was done by the investigators. BP was measured with mercury manometer (to the nearest 2 mmHg) on the right arm in the supine and standing positions after 5 min rest. Diastolic BP (DBP)-phase 5 was used. Heart rate (HR) was determined by pulse palpation. With this examination 15 persons were classified as WHO stage I, while 9 showed signs of left ventricular hypertrophy or eye-ground changes as in WHO stage II.

Design of the study

The antihypertensive effect of propranolol and practolol was evaluated in double-blind cross-over trial (Fig. 1). In the first treatment period the patients were given either propranolol ($n=10$) or



Fig. 1. Design of the study

The doses of propranolol and practolol were chosen in the ratio 1/2.5 corresponding to the known β -receptor blocking effect of the two drugs (4). Thus propranolol was given in a dose of 40 mg three times daily for one week and then 80 mg three times daily for seven weeks. Practolol was given in a dose of 100 mg three times daily for one week and 200 mg three times daily for seven weeks. After another four weeks on placebo treatment those who had previously been on propranolol treatment were crossed over to practolol and vice versa. BP in the supine and standing positions and HR were measured at 0, 8, 12, and 20 weeks. At each visit standardized questionnaire concerning side-effects was answered. After each treatment period the tablets were counted. Those who had taken less than 90% of the prescribed number were excluded.

RESULTS

Table I shows mean BP and HR before and after treatment for two months with propranolol and practolol for all 4 patients: 10 starting with propranolol and 14 with practolol.

1) Both drugs caused a significant reduction of P and HR ($p < 0.001$). 2) Both drugs caused an almost identical reduction of BP. 3) Propranolol lowered HR significantly more than practolol ($p < 0.01$).

Fig. 1 presents the mean BP and HR before and after 8, 12 and 20 weeks treatment for those starting with propranolol and practolol respectively.

1) Ten patients started with propranolol and 14

with practolol. No initial BP differences between the two groups were noted but those in the practolol group had slightly higher mean BP values. 2) After 8 weeks of treatment those starting with practolol showed a significant BP reduction (SBP and DBP $p < 0.001$). The smaller group ($n = 10$) starting with propranolol did not show a significant BP reduction. No BP difference between the two groups was noted. Both propranolol ($p < 0.001$) and practolol ($p < 0.01$) lowered HR significantly but propranolol somewhat more ($p < 0.01$). 3) After 4 weeks of placebo treatment BP and HR rose towards pretreatment values. There was no statistical difference compared with initial SBP, DBP and HR except for DBP in the practolol group. 4) Eight weeks treatment after cross-over produced almost identical BP reductions in the two groups. SBP and HR were significantly reduced ($p < 0.001$) compared with pretreatment and placebo values for both groups. DBP was significantly reduced ($p < 0.001$) for the propranolol group only.

Good BP control (SBP < 160 , DBP < 100) was achieved with propranolol treatment in 19/24 patients and with practolol treatment in 17/24. Four of the patients were non-responders to both drugs.

Table II shows the effect of propranolol and practolol on 10 patients with DBP ≥ 110 mmHg. Both drugs lowered BP significantly with no significant difference between the two groups.

Table I. Supine BP and HR for the total material before and during propranolol and practolol treatment and reductions of BP and HR (Δ)

	Systolic BP (mmHg)			Diastolic BP (mmHg)			HR	
	\bar{x}	Δ		\bar{x}	Δ		\bar{x}	Δ
Before treatment	173	17	—	106	7	—	82	13
During propranolol treatment	156	20	17	96	10	10	63	18
During practolol treatment	155	18	18	97	8	9	69	13

	I		II		III		IV	
SBP	170	18	160	18	160	17	165	20
DBP	104	7	98	8	104	8	98	9
HR	85	14	83	7	85	16	87	11
0								
20 week								
Practolol								
Placebo								
Practolol								
SBP	175	18	156	18	168	16	162	19
DBP	104	7	98	12	103	8	95	10
HR	81	13	70	10	80	12	83	12
0								
20 week								
Practolol								
Placebo								
Practolol								

Fig. 2 Supine systolic (SBP) and diastolic (DBP) BP and HR before and during propranolol and practolol treatment.

Side-effects

No patient developed signs or experienced symptoms of left ventricular failure or bronchial obstruction. No sign of orthostatic hypotension was found. Neither interruption of treatment nor dose reduction were needed. The adverse effects were few and transient. On propranolol treatment one patient had sleep disturbances and two felt dryness of the mouth. One experienced initial vertigo and one had transient diarrhoea. On practolol treatment one patient felt malaise and two had cold extremities. No significant changes in serum electrolytes, creatinine or uric acid were noted. No patient developed glucosuria or proteinuria.

DISCUSSION

The material in this study consisted of patients with mild to moderate hypertension. The high

drop-out rate might be explained by the somewhat strict criteria for cooperability.

The design of the study was planned with an intermediate placebo period in order to have the patients back to their pretreatment BP levels. HR returned to pretreatment values in both groups. BP did not in those starting with practolol. Although this might favour propranolol in the overall comparison, this slight difference should not change the conclusions drawn.

The results showed that practolol seemed to have the same BP-lowering effect as propranolol in these patients with mild to moderate hypertension (Table I and Fig. 2) and even among those with BP above 110 mmHg (Table II). Only moderate BP reduction was achieved in contrast to other authors (5, 11, 15). These differences are probably explained by different compositions of the groups studied as well as differences in doses

Table II Effect of 2 months practolol and propranolol treatment on BP and HR for 10 subjects with untreated DBP ≥ 110 mmHg

	Untreated			After propranolol			After practolol		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
184	170	84	158	100	60	160	100	76	176
162	110	76	134	94	66	142	90	68	168
192	114	70	154	94	68	174	104	58	178
174	116	100	154	98	56	148	100	78	174
178	110	77	140	90	58	144	96	64	174
196	110	68	178	108	56	188	106	54	174
200	110	60	192	11	56	182	106	66	174
184	118	70	174	112	60	170	104	54	174
166	11	77	154	96	58	146	98	68	174
200	116	80	176	108	86	160	90	94	174
Mean	184	112	75	161	101	67	161	99	68
S.D.	13	3.1	10.9	18.1	8.0	9.1	16.5	13	10.9

administered. The higher BP on practolol treatment is probably due to the intrinsic sympathetic effect of practolol ().

Fewer side-effects such as bradycardia, bronchial obstruction, sleep disturbances or peripheral arterial insufficiency can be expected during treatment with practolol than with propranolol. Side-effects, however, cannot be compared in this study because of the small number of patients.

In mild and moderate hypertension practolol seems to have the same BP-lowering action as propranolol. Whether fewer side-effects occur must be further investigated.

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CHRONIC INTERSTITIAL PNEUMONITIS WITH FIBROSIS DURING LONG TERM TREATMENT WITH NITROFURANTOIN

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Abstract Chronic pulmonary lesions in conjunction with prolonged nitrofurantoin treatment has occurred in 15 patients with symptoms in the form of dyspnoea, cough fever and sometimes loss of weight. The onset was insidious after 6 months to 6 years of continuous or intermittent treatment. X-rays of the lungs showed streaky or patchy infiltration bilaterally most pronounced basally. Spirometry revealed restrictive ventilatory reduction in 8 of 10 patients. The time during which the patients continued with nitrofurantoin after the onset of symptoms appears to be important but not decisive for the prognosis. Observation of symptoms from the respiratory tract and regular X-rays during nitrofurantoin treatment are recommended.

Acute pulmonary hypersensitivity reactions during treatment with nitrofurantoin are now well known. The Swedish Adverse Drug Reaction Committee received more than 150 reports from 1966 to June 1973. The adverse reactions characteristically appear in acute form after 1-3 weeks of treatment—after a shorter time if the patient has received nitrofurantoin earlier. The symptoms consist of fever, dyspnoea, leukocytosis and eosinophilia. More than half of the patients have cough. In about 80% of cases radiological lesions are seen, such as pulmonary infiltrates and pleural effusion. The symptoms recede within a day or more after the cessation of treatment.

Reports of chronic interstitial pneumonitis with fibrosis are considerably less common. In 1966 the first report was published of a patient who after 1 year of treatment with nitrofurantoin, reacted with severe dyspnoea and cyanosis (9). Since then another 11 cases of pulmonary reactions of chronic type have been reported (2, 3, 4, 5, 6, 7, 10, 11). Although this side-effect is yet little known, we considered it of interest to publish the reports received by the

Swedish Adverse Drug Reaction Committee placed at our disposal by the reporting physicians.

MATERIAL

The material comprises 15 reports of interstitial pneumonitis (with fibrosis) received by the Committee from 1970 to Nov 1973. The causal connection with nitrofurantoin treatment has been judged to be probable or not excluded (12).

RESULTS

Basic data concerning the patients' pulmonary diseases are presented in Table I. Table II shows the nitrofurantoin dose, the duration of treatment before and after the onset of symptoms, cortisone treatment, if any, converted to equivalent dose of prednisolone, observation period after withdrawal of nitrofurantoin, and particulars of the subsequent course.

Most patients were women aged 60-70 years who had been continuously or intermittently treated with nitrofurantoin over a lengthy period. They do not appear to have been overdosed from the point of view of renal function. Symptoms in the form of dyspnoea, cough, fever and sometimes loss of weight, usually had an insidious onset after 6 months to 6 years of treatment. In some cases the onset occurred in conjunction with cold. In two cases the clinical symptoms were preceded by radiological disturbances. In all patients X-ray of the chest showed pulmonary lesions in the form of streaky and patchy infiltrations bilaterally, usually most pronounced basally. On occasions there was also pleural effusion. In 8 of 10 patients spirometry revealed a restrictive ventilatory reduc-

Table 1 Clinical data

Medical record	
Solely urinary tract infection	7
Joint symptom	3
Joint symptoms+other disease	1
Other diseases	4
Lung X-ray	
Infiltrate	15
Infiltrate and pleural exudate	3
Spirometry	
Restrictive	8
Normal	
Not performed	5
Lung biopsy	
Fibrosis	4
Not performed	11
Serum pentamine (mg/100 ml)	
<1.0	7
>1.0	
Not indicated	6

tion. All patients had an elevated ESR in three cases more than 100 mm/h (Westergren), two had around 600 eosinophilic granulocytes/ μ l. Serological reactions were recorded in several patients.

Case 1

A 71-year-old woman, hospitalized 4 years earlier for hyperthyreosis and suspected collagenosis. At the time of the present attack she had been treated with Alzet (methidopa) for 1 year. She fell ill with cough, no day-night fever and dyspnoea. X-ray of the chest showed pleural fluid bilaterally and an abundance of distinctly delimited parenchymal infiltrates within large portions of the right and the basal portion of the left lung. The symptoms were new since her stay in hospital 4 years previously. Spirometry revealed restrictive ventilatory reduction, ESR 125 mm/h, LDH 6.5 mU/l, ANF positive to dilution 1/100, γ -globulin 2.5 g/100 ml, rheumatoid factor, Coombs and Mantoux tests negative. She was not treated with corticosteroids. She was asymptomatic 18 months after the withdrawal of nitrofurantoin; the radiological symptoms had regressed, spirometry showed normal conditions, ESR 60 mm/h, ANF 1/25 and LDH had become normalized.

Another two patients had a negative tuberculin test, six a rise of serum γ -globulin and two ANF above 1/100. In one patient (no. 3) with positive LE cell phenomenon this persisted after the withdrawal of nitrofurantoin.

Case 3

A 71-year-old woman, earlier generally healthy, fell ill with dyspnoea after a cold. Her condition gradually de-

veloped with increasing dyspnoea, cough and fever. Erythromycin had no effect. The right lung X-ray showed extensive diffuse hazy partially confluent infiltrates centrally and within most of the middle lobe around and connected with the hilar area. In the left lung there were thin hazy infiltrations outside the hilar area and basally. LE cell $\times 3$ were positive. Mantoux negative. She was treated with Betapred (betamethasone), 0.5 mg $\times 3$ and Tetralysal (tetracycline). At examination 6 weeks after withdrawal of nitrofurantoin she was asymptomatic and pulmonary X-ray showed almost complete recovery. At regular check-ups during the following year the pulmonary picture was normal but she still had a few LE cells.

Liver reaction concurrently with pulmonary symptoms occurred in one patient (no. 4).

Case 4

A 58-year-old man, earlier generally healthy, fell ill with jaundice without respiratory tract symptoms. Nitrofurantoin was withdrawn and the pathological liver function tests became normalized. After 10 days nitrofurantoin was recommenced whereupon SGOT and SGPT rose again. After 1 week of nitrofurantoin treatment he developed fever and catarrhal symptoms. Pulmonary X-ray showed fairly extensive streaky and patchy parenchymal infiltration basally bilaterally and minor infiltrations in the middle portions of the lungs, SGOT 570 mU/l, SGPT 410. He was not treated with corticosteroids. 10 months after the withdrawal of nitrofurantoin the pulmonary X-ray showed unchanged status. The liver function tests were normal. At examination after 9 months he was subjectively healthy and the X-ray symptoms had strikingly improved.

Two other patients had somewhat elevated SGOT and SGPT values. One patient (no. 7) had had hepatitis of unknown genesis when the respiratory symptoms started. At examination by reason of the pulmonary lesions 1 year later the SGOT and SGPT values were normal.

Lung biopsy was performed on 4 patients. The findings are illustrated by the following case.

Case 5

A 61-year-old woman who had occasionally been troubled by a mild cardiac insufficiency and by ache and swelling in hands and knees. She was digitized and used salicylates regularly. Fell ill with increasing dyspnoea followed later by fever and cough. Various antibiotics were without effect. Pulmonary X-ray showed diffuse infiltrates bilaterally and spirometry restrictive ventilatory reduction. Lung biopsy revealed considerable increase of interstitial connective tissue. The contracted flattened alveoli were coated with slightly atypical dark-celled epithelium. In the connective tissue there were few lymphocytes and occasional plasma cells. ANF was positive 1/128. She was not treated with corticosteroids. At examination 18 months after withdrawal of nitro-

Table II Nitrofurantoin dose and duration of treatment cortisone treatment observation period and course after withdrawal of nitrofurantoin

Pat. no.	Age (y)	Sex	Nitrofurantoin dose (mg)	Duration of treatment		Prednisolone dose (mg)	Obs. period	Course	
				Before symptoms	After symptoms			Subjectively	Radiological impression
1	71	♀	50x3	11 mo.	mo	None	1 y 6 mo	Satisfactory	III
2	64	♀	5x	4 y	7 mo	5 3	7 mo	Satisfactory	R. pres
3	71	♀	?	3 y 6 mo	4 mo	5 3	1 y	Satisfactory	Norm
4	58	♂	30x2	7½ mo	½ mo	None	9 mo	Satisfactory	Unchanged
5	61	♀	?	Several y	5 mo.	None	1 y 6 mo	Improved	I mo
6	74	♀	50 3	At least 1 y	1 y 3 mo	None	1 y	Satisfactory	Regressed
7	87	♀	50x4	6 mo	1 y	5	10 mo.	Improved	Some regression
8	6.	♀	?	6 y	4 y	5 4	3 y	Death	Progressive
9	84	♀	?	Prolonged	mo	None	3 mo.	Improved	Regression
10	54	♀	50 2	6 mo	6 mo.	5x3	mo	Improved	Unchanged
11	48	♂	50x	1 y 3 mo.	3 mo	None	5 mo	Satisfactory	Reg.
12	69	♀	50x	1 y 3 mo	3 mo	None	½ mo	Improved	Regression
13	62	♀	?	4 y	3 y	Unknown	y 3 mo	Improved	Unchanged
14	89	♀	50 3-1	6 mo.	mo	40 1	6 mo	Improved	Regression
15	72	♂	50 3	2 y., 3 mo	3 mo	10 3	2 y 3 mo	Unimproved	Unchanged

function she was subjectively improved and the lung X-ray as largely normal. Rheumatoid factor tests and tests for ANF are negative.

Course

Nitrofurantoin treatment was discontinued in all cases. Seven patients were given no further treatment. All improved subjectively and radiologically. Eight patients received corticosteroids generally in low dosages. Six of them improved but two had an unchanged radiological picture on follow-up after months and 14 years respectively. Of the remaining two patients the condition of one (no 15) remained unchanged both subjectively and objectively while the other (no 8) died from respiratory and secondary circulatory insufficiency.

Case 8

A 6-year-old woman who had had dry bilateral pleuritis in youth. She fell ill with cough and in due course dyspnoea. Pulmonary X-ray showed bilaterally basally extensive streaky and patchy infiltrations with thinner portions, possibly emphysematous pockets. Lung biopsy revealed fine bronchiectases, chronic bronchitis, chronic perimontitis and light interstitial fibrosis. She was treated with Celestalon (betamethasone), 0.5 mg 2-4 times intermittently during the following 7 years, and with tuberculostatics. The nitrofurantoin treatment was continued for at least 4 years after the onset of symptoms. During this time her condition improved subjectively but the radiological picture remained much the same. Thereafter both subjective and radiological improvement occurred and she died 7 years after the onset of symptoms from

respiratory insufficiency with cardiac insufficiency. Contributory cause: Pulmonary X-ray just prior to death had shown pronounced streakiness throughout the entire pulmonary parenchyma as pulmonary fibrosis. Spirometry revealed pronounced ventilatory reduction of restricted type. Autopsy was not performed.

The patients treated with steroids continued to receive nitrofurantoin for on an average 14.8 months after the onset of symptoms compared with 6 months for those not given steroids.

DISCUSSION

Chronic interstitial pulmonary lesions with fibrosis may occur in several different conditions. Hamman-Rich syndrome is accompanied by an acute or chronic diffuse interstitial pulmonary fibrosis. The lesions commence with an interstitial oedema, exudate in the alveoli and capillary damage. An increasing fibrosis develops interstitially, the alveoli become coated with a cuboidal epithelium which desquamates and the alveoli are obliterated. Dilation of the bronchioli may cause cysts and give the lungs a honeycombed appearance. Lung function studies show ventilatory reduction of restricted type and obstacles to diffusion. Radiological symptoms appear only in a fairly late stage in the form of diffusely circumscribed dense fine patches usually basally. The cause of the Hamman-Rich syndrome is unclear. Interstitial pneumonitis, fibrosis similar to that in Hamman-Rich

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Other diseases	4
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A 58-year-old man, earlier generally healthy, fell ill with jaundice without respiratory tract symptoms. Nitrofurantoin was withdrawn and the pathological liver function tests became normalized. After 10 days nitrofurantoin was recommenced, whereupon SGOT and SGPT rose again. After a few weeks of nitrofurantoin treatment he developed fever and catarrhal symptoms. Pulmonary X-ray showed early extensive streaky and patchy parenchymal infiltrations basally bilaterally and minor infiltrations in the middle portions of the lungs. SGOT 570 mU/l, SGPT 410. He was not treated with corticosteroids. 7 months after the withdrawal of nitrofurantoin the pulmonary X-ray showed unchanged status. The liver function tests were normal. At examination after 9 months he was subjectively healthy and the X-ray symptoms had strikingly improved.

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In the present material the prognosis was comparable to that reported in the literature. Half of the patients had been treated with cortisone, half had received no treatment. The prognosis was rather worse in the former group. This is probably due not to the cortisone treatment per se, but to the fact that the former group had been treated with nitrofurantoin for a lengthy period after the onset of symptoms and had more advanced pulmonary lesions.

CONCLUSION

From the material presented it is apparent that pulmonary lesions may be caused by current doses of nitrofurantoin. The prognosis seems to be worse the longer the patients have been treated after the onset of symptoms, but there may be residual lesions even after brief treatment. Patients on prolonged or repeated nitrofurantoin treatment should be regularly checked for symptoms from the respiratory tract and for fever. As the clinical symptoms may be preceded by radiological disturbances, long X-rays should be checked say every six months.

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has been elicited by busulfan hydralazine and combined hydralazine and hexamethonium (1).

Collagenoses may cause similar lesions to those described by Hamman-Rich (8). In rheumatoid arthritis pleural exudate is the commonest pulmonary symptom. Diffuse interstitial fibrosis may also occur appearing either before concurrently with or after the joint symptoms. In fully developed systemic lupus erythematosus diffuse fibrosis is seldom seen but pleural exudate is common.

The pulmonary lesions in varicella may develop into a chronic form with interstitial fibrosis. If one can follow the X-rays from the acute phase one sees how the hilar lymphoma diminishes in extent and the fine-patchy parenchymal infiltrates become increasingly coarse and confluent. At a later stage this leads to fibrosis and general emphysema (8).

Among the patients reported to the Adverse Drug Reaction Committee seven had no disease apart from infection of the urinary tract and had received no other drugs which might have a significance for the pulmonary lesions. These cases might possibly be assigned to the Hamman-Rich syndrome. The improvement which regularly occurred after cessation of the nitrofurantoin treatment suggests a causal connection between the pulmonary changes and this drug.

Of the other eight patients cases 1, 5 and 13 had joint complaints before and/or during the disease. The rheumatoid factor test was not positive.

Cases 1 and 5 received no corticosteroid. Nevertheless the ANF titre fell or became negative and the patient improved. In these cases nitrofurantoin must be considered to be the most probable aetiology of the pulmonary lesions (case 1 had at the same time been treated with Aldomet® (methyldopa) but had a negative Coombs' test). Case 13 had had pain in the joint for many years with slight swelling of the interphalangeal joints. Her lung disease improved objectively but not radiologically during the cortisone treatment. This patient, however, had been on nitrofurantoin for 3 years after the onset of symptoms. It must be considered probable that the lengthy nitrofurantoin treatment caused the pulmonary symptoms.

Case 2 had an impaired renal function and secondary anaemia. She had received repeated blood transfusions and developed a siderochrestic anaemia. Her pulmonary symptoms had from the outset been attributed to the uraemia but on the other

hand the serum creatinine was only moderately elevated (7.9 mg/100 ml) and on the other the radiological symptoms did not accord with those often found in uraemia (8).

Case 8 had a mild bronchial asthma and diabetes mellitus. She improved objectively under steroid treatment. In case 9 there was a cardiac component which may have been a contributory factor. Case 10 had an apical tuberculosis but the time relation with the nitrofurantoin treatment renders it probable that the latter was the cause of the lesions. Case 14 had joint symptoms and diabetes mellitus which was treated with Diabinese (chlorpropamide) and Dibein® Retard® (phenformin). Here again there was a close relationship in time between the pulmonary symptoms and the nitrofurantoin treatment.

Twelve cases of chronic pulmonary reactions to nitrofurantoin have so far been reported in the literature (2, 3, 4, 7, 9, 10, 11). One further case is to be published (6). The present cases accord with those previously reported in respect of nitrofurantoin dosage, duration of treatment before and after onset of symptoms, clinical picture, lung X-ray and spirometry findings. But in contrast to most of the previously reported cases the present patients usually had fever at the onset of symptoms.

As in the present cases, earlier published cases had a negative tuberculin test (4, 7, 9) and rise of γ -globulin (3, 7, 9, 11). Unfortunately LDH was investigated only in three of the present patients, one of whom had an elevated value. In the literature a rise of LDH is reported in four patients (3, 7).

The histological picture from lung biopsy (4 patients) also accorded with reports in the literature (2, 3, 5, 7). Israel et al. (3) examined such lung tissue also in respect of antibody-antigen complexes by immunofluorescence technique with negative result.

Most of the patients reported in the literature improved on cortisone treatment after the withdrawal of nitrofurantoin. One patient, however, who had received nitrofurantoin over a period of 6 years, 3 of which after the onset of symptoms, had unchanged lung function and radiological picture at follow-up. One patient who had also received nitrofurantoin for several years after the onset of symptoms died. This case has not yet been published (6).

TRACE ELEMENTS IN SERUM AND URINE FROM HYPERTENSIVE PATIENTS TREATED FOR SIX MONTHS WITH CHLORTHALIDONE

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Abstract. Urinary excretion and serum concentration of antimony, arsenic, bromine, cadmium, caesium, cobalt, copper, gold, iron, mercury, molybdenum, rubidium, scandium, silver, tungsten and zinc from 6 hypertensive patients before and after 6 months treatment with chlorthalidone have been investigated. The method consisted in neutron activation analysis combined with recently developed ion exchange technique. A previously observed increase in urinary excretion of bromine, cadmium, cobalt, rubidium and zinc after short-term treatment with chlorthalidone was also present after long-term treatment with regard to rubidium and zinc and possibly also to bromine, cadmium and cobalt. A significantly lowered concentration in serum after long-term treatment was found only for rubidium,

molybdenum, rubidium, scandium, silver, tungsten and zinc from hypertensive patients treated with chlorthalidone for 6 months.

MATERIAL AND METHODS

Six patients, 4 men and 2 women ranging age from 28 to 60 years, with mild to moderate hypertension treated with chlorthalidone in daily dose of 50 mg per os for 6 months were subjected to the study. BP on admission varied between 175/105 in the youngest patient and 260/150 in one of the women. The heart rates varied between 360 and 700 cm^2/m^2 and pulse of the patients had glomerular filtration rate lower than 70 ml/min . Proteinuria or bacteriuria did not occur in any case. All subjects had previously been investigated with respect to the trace element concentration in serum and urine before treatment (19).

Blood samples, taken in fasting state, were drawn through plastic catheters inserted into cubital vein and the first 10 ml of blood was discarded. After centrifugation 1 ml samples of serum were pipetted into quartz ampoules, which were rapidly flame-sealed as described previously (20). On the same day urine was collected for 24 hours in thoroughly cleaned polythene bottles. Samples of 1-2 ml of urine were pipetted into quartz ampoules, which after flame-sealing were ready for irradiation. The quartz ampoules were irradiated together with standards in the R2 reactor at Studsvik at thermal neutron flux of $2 \cdot 10^{16}$ $\text{n}/\text{cm}^2/\text{sec}$ for 24 hours. The chemical separation procedure and the γ -spectrometric measurements and identification of the elements have been described elsewhere (1, 12, 18).

Statistical methods

In the statistical analysis Student's *t*-test has been used in comparing serum concentrations and the paired *t*-test in comparing urinary excretions. The degrees of significance obtained have been expressed as: - not significant ($p > 0.05$), almost significant ($0.01 < p < 0.05$), significant ($0.001 < p < 0.01$), ** highly significant ($p < 0.001$).

Earlier investigations (7, 15, 16) have indicated a possible connection between trace elements and arterial hypertension. In a previous study (19) the serum concentration and urinary excretion of 17 trace elements from hypertensive patients were found not to differ significantly from those of normotensive subjects. However, during treatment of the hypertensive patients with chlorthalidone (Hygroton®) a raised urinary excretion of bromine, cadmium, cobalt, rubidium and zinc was observed. The serum concentration after 5 days treatment was only decreased with respect to rubidium and the question arose whether the increased urinary excretion of these elements was only a temporary phenomenon or whether it would continue during treatment and possibly cause a deficiency of some of the trace elements.

The aim of the present study was to investigate the serum concentration and the urinary excretion of antimony, arsenic, bromine, cadmium, caesium, cobalt, copper, gold, iron, mercury,

Table I 24-hour urinary excretion of trace elements with known biological function before and after 6 months treatment with chlorthalidone ($\mu\text{g}/24 \text{ h}$)

Case no.	Co		C		Fe		Zn	
	Before	After	Before	After	Before	After	Before	After
1	0.44	0.6	28	46	51	70	310	440
	1.8	0.74	37	45	130	46	350	410
3	1.7	0.94	37	76	160	180	460	570
4	0.45	0.77	37	38	49	140	430	1 020
5	0.67	1.8	4	35		170	140	660
6	0.59	1.6	77	49	67	110	800	1 035
Mean \pm S.D.	0.94 \pm 0.63	1.1 \pm 0.30	37 \pm 24	48 \pm 15	93 \pm 49	121 \pm 55	420 \pm 220	690 \pm 280
Mean difference	+0.14		+1		+19		+274	

RESULTS

The results of trace element analysis of the 24-hour urinary samples collected after 6 months treatment with chlorthalidone are given in Tables I-III. In these Tables as well as in Tables IV-VI the trace elements have been divided into those with known (Table I), suspected (Table II), and without known biological function (Table III).

Among the trace elements with known biological function zinc was present in an almost significantly higher amount in the urine after 6 months treatment than before treatment and among the trace elements with suspected biological function the rubidium excretion was almost significantly higher after treatment. The urinary excretion of bromine, cadmium and molybdenum after 6

Table II 24-hour urinary excretion of trace elements with suspected biological function before and after 6 months treatment with chlorthalidone ($\mu\text{g}/24 \text{ h}$)

Case no.	Br		Cd		Mo		Rb	
	Before	After	Before	After	Before	After	Before	After
1	900	3 300	1.9	1.5	33	78	1 600	2 400
2	100	3 400	1.8	2.4	89	250	000	200
	5 400	9 800	0.78	3.2	87	200	600	800
	3 700	8 900	0.70	0.70	87	150	3 300	5 500
	1 700	5 700	0.54	4.3	34	40	2 000	4 000
	1 700	6 400	1.0	2.7	95	95	3 300	3 100
\pm S.D.	1 700	1 400	1.1 \pm 0.99	5 \pm 1.3	71 \pm 29	170 \pm 73	900 \pm 700	3 400 \pm 1 200
Mean difference	-1 000		+1.4		99		+900	

Table III 24-hour urinary excretion of trace elements without known biological function before and after 6 months treatment with chlorthalidone ($\mu\text{g}/24 \text{ h}$)

Case no.	Ag		As		Au		C	
	Before	After	Before	After	Before	After	Before	After
1	0.32		230	17	0.00058	0.0071	8.8	12
2	1.1	0.11	120	98	0.0058	0.00070	8.1	15
3	0.43	0.5	370	95	0.0010	0.00056	14	8.7
4	0.46	0	570	260	0.083	0.0017	17	26
5	0.87	0.76	58	7	0.0020	0.0058	13	5
6	0.40		110	1 300	0.00052	0.00088	14	4
Mean \pm S.D.	0.60 \pm 0.31	0.46 \pm 0.4	40 \pm 200	310 \pm 300	0.016 \pm 0.033	0.0035 \pm 0.0040	13 \pm 3	19 \pm 7
Mean difference	-0.20		+43		-0.01		+5.4	

months treatment was also high but not significantly higher than before treatment. None of the trace elements without known biological function showed significantly increased or decreased excretion after 6 months treatment.

The mean values and standard deviations of the trace element concentrations in serum before and after 6 months treatment are given in Tables IV-VI. None of the 17 trace elements investigated, with the exception of rubidium showed any significant difference in serum concentration before and after 6 months treatment. The values for cadmium, caesium, cobalt, gold, mercury and scandium were low after treatment while copper and molybdenum values were high. These differences were however not significant.

DISCUSSION

In a previous study of trace elements in serum and urine from hypertensive patients before and after 5 days treatment with chlorthalidone (19) highly significantly increased amounts of bromine

rubidium and zinc and almost significantly increased amounts of cobalt and cadmium were observed during treatment. However the serum concentration of only rubidium was significantly lower after 5 days treatment. If the increased urinary excretion of some trace elements found during short-term treatment were to continue during treatment for longer periods, one would expect that deficiency might develop. In the present study after 6 months treatment high urinary excretion and low serum concentrations were observed for some of the elements investigated. In the following discussion the elements will be dealt with in the groups used earlier, i.e. trace elements with known suspected and without known biological function.

Trace elements with known biological function

Cobalt The serum concentration was lower and the urinary excretion higher after 6 months treatment than before treatment but the differences were not significant. An almost significant increase in urinary excretion during 5 days treatment has previously been observed (19) and it is possible that too few cases were investigated in the present study to obtain significant differences.

Copper The mean serum concentration of copper after 6 months treatment was high (1.07 µg/ml) as compared to before treatment (0.80 µg/ml) and to the concentration after 5 days treatment (0.84 µg/ml). The difference between 1.07 and 0.80 was however not significant. The mean value after 3 months treatment almost the same as the mean value previously found in serum from 8 healthy individuals. The urinary excretion of copper after 3 months treatment was not significantly changed.

Se	
Before	After
28	3
37	40
33	
41	44
46	31
44	54
32-51	38 ± 1
	6.4

Hg	
Before	After
0.58	0.4
1.0	0.74
1.18	0.79
0.99	8
1.16	
0	0.91
65 ± 0.41	1.0
0.48	

Sb	
Before	After
0	0.71
1.8	1.6
1.4	2.1
1	1.1
1	1.2
1	1.6
1.9 ± 0.2	1.6 ± 0.24
1.7	

Sc	
Before	After
0.040	0.0075
0.011	0.0084
0.015	0.0069
0.0049	0.009
0.16	0.076
0.0039	0.0043
0.083 ± 0.14	0.019 ± 0.028
0.061	

W	
Before	After
9.7	1
6	15
1	1
44	41
1	1
21	1.8
13.1	70.24

Table IV Serum concentration of trace elements with known biological function before and after treatment with chlorthalidone (mean \pm S.D. $\mu\text{g/ml}$)

	Untreated	After 6 months treatment
C	0.00044 \pm 0.00015 (n=5)	0.00036 \pm 0.00016 (n=5)
Co	0.80 \pm 0.31	1.07 \pm 0.27
Fe	1.26 \pm 0.53	1.37 \pm 0.74
Zn	0.91 \pm 0.11	0.83 \pm 0.12

Iron No marked changes in the iron concentration in serum or urine in relation to treatment with chlorthalidone were observed.

Zinc The amount of zinc in urine after 6 months treatment was almost significantly higher than before treatment. The mean value of 690 $\mu\text{g}/24$ h was almost as high as that (720 $\mu\text{g}/24$ h) found after 5 days treatment which was highly significantly different from the mean value of 520 $\mu\text{g}/24$ h before treatment. Thus the previously observed increase in zinc excretion after short-term treatment persists after long-term treatment. However the serum concentration after 6 months treatment was not significantly lower than before treatment. Enhancement of renal excretion of zinc by hydrochlorothiazide has recently been observed by Pak et al. (6). They found that the increased urinary zinc excretion also persisted after 1 month's treatment and that zinc concentration in serum was unchanged or within the upper range of normal after treatment for 1 year or more.

Trace elements with suspected biological function

Bromine The urinary excretion of bromine was but not significantly higher after 6 months treatment than before treatment. If the mean

Table V Serum concentration of trace elements with suspected biological function before and after treatment with chlorthalidone (mean \pm S.D. $\mu\text{g/ml}$)

	Untreated	After 6 months treatment
Br	3.8 \pm 1.5	3.8 \pm 1.7
Cd	0.0018 \pm 0.0018	0.0013 \pm 0.0014
Mo	0.0049 \pm 0.0054	0.010 \pm 0.005 (n=2, 15)
Rb	0.24 \pm 0.03	0.17 \pm 0.02 (n=4, 03)
Se	0.17 \pm 0.05 (n=4)	0.18 \pm 0.07 (n=4)

Table VI Serum concentration of trace elements without known biological function before and after treatment with chlorthalidone (mean \pm S.D. $\mu\text{g/ml}$)

	Untreated	After 6 months treatment
Ag	0.61 \pm 0.18	0.55 \pm 0.24
As	5.9 \pm 5.1	6.7 \pm 9.4
Au	0.0041 \pm 0.0036	0.0017 \pm 0.0028
Cs	1.5 \pm 0.88	1.1 \pm 0.58
Hg	2.3 \pm 1.4	1.7 \pm 1.2 (n=5)
Sb	0.71 \pm 0.77	0.77 \pm 0.36
Sc	0.15 \pm 0.21	0.011 \pm 0.013
W	5.2 \pm 5.3	11 \pm 14

value for urinary bromine excretion in 15 untreated hypertensive patients previously investigated is compared to the mean value for the urinary excretion of bromine from the 8 patients after 6 months treatment an almost significant increase in excretion is found for the latter group. However the concentration of bromine in serum was almost the same after 6 months treatment as before.

Cadmium The urinary excretion of cadmium after 6 months treatment was high. The paired mean difference between the excretion before and after 6 months treatment was not significant but the *t* value was 2.0. Calculation with Student's *t*-test showed an almost significant difference. After 5 days treatment an almost significant rise in urinary excretion was previously observed. The serum concentration after 6 months treatment was low but not significantly lower than before treatment.

Molybdenum A high mean value of molybdenum was noted in serum after 6 months treatment, but as compared to the mean value before treatment there was no significant difference. The urinary excretion after 6 months treatment did not differ significantly from that before treatment when calculated on paired mean differences. Calculation with Student's *t* test however showed a significantly higher excretion after treatment.

Rubidium After short-term treatment with chlorthalidone a highly significant increase in the urinary excretion and an almost significant decrease in the serum concentration of rubidium as compared to before treatment have previously been found (19). After 6 months treatment the urinary excretion was also high and differed almost significantly from that before treatment, and

the serum concentration was highly significantly decreased as compared to before treatment. Rubidium usually behaves in the same way as potassium. However all the patients treated for a long time with chlorthalidone received potassium chloride in addition and no hypokalaemia was noted.

Selenium No marked changes in serum concentration or urinary excretion of selenium in relation to treatment with chlorthalidone were noted.

Trace elements without known biological function

The variability in serum concentration and urinary excretion of the trace elements without known biological function was large. In the previous study the serum concentration and urinary excretion after short-term treatment with chlorthalidone was compared to that before treatment; a significant difference was only obtained for caesium which showed a decreased urinary excretion after treatment. When the values after long-term treatment with chlorthalidone were compared to those before treatment, no significant differences were obtained for any of these elements.

In conclusion the previously observed increase in urinary excretion of bromine, cadmium, cobalt, rubidium and zinc after short-term treatment with chlorthalidone was also present after long-term treatment with regard to rubidium and zinc and possibly also to bromine, cadmium and cobalt. However a significantly lowered concentration in serum after long-term treatment was only found for rubidium.

Whether a lowered concentration of rubidium in the serum has any pathological significance in human beings remains uncertain. That the action of rubidium on the contraction of the isolated frog's heart was almost identical with that of potassium was observed already by Ringer (11). Furthermore the same influence has been found from the extracellular ionic concentration of potassium and rubidium on the resting potential, nerve and muscle preparations and on the configuration of the ECG (10). Thus it may be possible that a lack of rubidium may have similar effects to lack of potassium or at least potentiate the effect of an already developed hypokalaemia.

The increased urinary excretion of zinc was not accompanied by a significantly decreased concentration in serum which may be explained either by increased gastrointestinal absorption or by mobiliza-

tion from tissues. If a tissue zinc deficiency develops during long-term treatment with chlorthalidone and hydrochlorothiazide it may be of clinical significance. Zinc is known to be an essential trace element. It constitutes a part of the molecule of several enzymes connected with carbohydrate and protein metabolism. Zinc deficiency is known to cause various pathological conditions in different animals (17). In man however the picture is not as clear. Dwarfism (9, 14) and impaired tissue repair (8, 13) have been reported as well as a connection with beri-beri, porphyria, postalcoholic cirrhosis and diabetes mellitus (2, 3, 4). Recently a rare skin disease acrodermatitis enteropathica has been reported to be cured by oral administration of zinc (5). The role of zinc in these various diseases is however not fully understood.

ACKNOWLEDGEMENTS

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ZINC PROTAMINE-GLUCAGON IN THE TREATMENT OF PAGET'S DISEASE OF BONE

PRELIMINARY REPORT

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Paget's disease of the bone is rarely painful. However the treatment of these rare cases has until recent years been poor.

A symptomatic and biochemical effect has been shown by treatment with mithramycin (4, 7) and actinomycin (5) but the toxicity of these drugs makes them unsuitable for treatment of ambulatory patients. Calcitonin has been reported to induce clinical and biochemical remission (2, 8, 9) but the half-life of the hormone is so short that at least 1 or 2 daily injections are required. A most striking and dramatic effect on the clinical and biochemical findings has been demonstrated during treatment with i.v. infusion of glucagon (3).

Due to the difficult administration of both calcitonin and glucagon and the effect of the latter we here report the biochemical and clinical results from a patient with Paget's disease of bone treated with zinc-protamine-glucagon with prolonged action.

METHODS

Total urinary hydroxyproline was measured by the methods of Bergman and Loxley (1). Serum alkaline phosphatase was measured by the method described by March et al. (6). Calcium in serum and urine by flame spectrophotometry.

CASE REPORT

A 70-year old man with intolerable pain in the femur and radiological and histological evidence of Paget's disease of bone was studied.

Initially serum calcium was 105 mg/l, plasma phosphate 28 mg/l and serum alkaline phosphatase 1.6 King Armstrong units/100 ml.

The patient, treated with zinc-protamine-glucagon (suggested and supplied by Nov. Industry A/S) 1 mg/day subcutaneously (one daily injection for 14 days) and thereafter 4 mg/day for the next 3 weeks. The treatment resulted in fall in urinary calcium total urinary hydroxyproline and serum alkaline phosphatase (Fig. 1). Serum calcium and plasma phosphatase were nearly unchanged during the therapy.

A dramatic effect—the bone pain occurred after initiation of the treatment. The night after the first injection the patient suffered no bone pain at all and he slept undisturbed for the first time in three months.

Rather pronounced side-effect occurred during the first series of treatment with 4 mg zinc-protamine-glucagon. His resting pulse rose from 60 to 80 beats/min, his BP rose from 120/80 to 170/90 mmHg and he experienced mental imbalance. These side-effects disappeared after interruption of the treatment.

Half year after the first series he had relapse of his pain in the left femur. He was treated with 1.25 mg zinc-protamine-glucagon for 8 days. Again his symptoms disappeared immediately and on this dose he had no side-effect.

DISCUSSION

Normalizing of the clinical and biochemical signs of Paget's disease of bone treated with zinc-protamine-glucagon has not previously been reported.

In our patient the treatment produced a reduction of serum alkaline phosphatase, total urinary hydroxyproline and 24-hour urinary calcium. Furthermore the patient experienced complete relief of bone pain. The results confirm the findings of Condon (3) who treated 4 patients with glucagon given by i.v. infusion.

Using zinc-protamine-glucagon only one daily

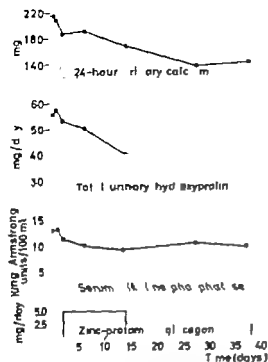


Fig 1 Level of 24-hour urinary calcium, total urinary hydroxyproline and serum alkaline phosphatase during treatment with zinc-prolamine-glucagon.

jection is required and that facilitates the administration of the therapy. The side-effects are considered to be negligible especially when only 1 mg/day is administered.

The presented results suggest that zinc-prolamine-glucagon may be useful in the treatment of Paget's disease of bone but further trials are clearly necessary.

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BONE MINERAL IN SARCOIDOSIS

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Abstract. The uptake of ^{85}Sr in the spine and both the bone mineral linear density (g/cm) and mineral density (g/cm^2) of the trabecular radius, midshaft radius and ulna have been measured in 21 untreated sarcoid patients using the ^{241}Am γ -transmission method. The results were compared with control subjects who showed no evidence of bone involvement or disturbances in calcium kinetics. Sarcoidosis was considered to be subacute active in 14 cases (5 male and 9 female), subacute inactive in four (1 male and 3 female) and chronic active in three (1 male and 2 female). The study showed, (a) In women the mean ^{85}Sr uptake in the spine was significantly smaller than in the controls. (b) In the early stages of the disease the ^{85}Sr uptake values were lower than when the disease was prolonged both in men and women. (c) The bone mineral linear density of the trabecular radius in active subacute sarcoidosis was significantly higher than in the controls. The relative difference in mineral density was of the same magnitude. (d) In chronic sarcoidosis neither the ^{85}Sr uptake in the spine nor the bone mineral values differed from the control values. (e) The mineral linear density and density in the midshaft radius and ulna were at the same level as in the controls. (f) A significant negative correlation was established between the ^{85}Sr uptake in the spine and the bone mineral density in the distal radius. It seems evident from this study that patients in the acute phase of sarcoidosis have an abnormally high bone mineral density and that there is distinct mineral decrease correlated with the duration of the disease.

Changes in calcium metabolism are known to occur in conjunction with sarcoidosis. The incidence of hypercalcemia has ranged from 0.7 to 6.5% in different studies (18, 28). With the exception of the materials reported by Rudberg-Roos (31) and Sharma et al (35) hypercalcemia has been rare in Scandinavia and Great Britain. In Finland elevated serum calcium values were established by Putkonen et al (30) in only 3.3% of 60 patients with sarcoidosis. Hypercalcemia has been found to be 2-7 times more common than

hypercalcemia (38). Changes in calcium metabolism are evidently more common in chronic and widespread forms of the disease (11). Since Schaumann (33) published his studies it was believed that disturbances of calcium metabolism are caused by the bone manifestations of sarcoidosis until it was observed that there was no correlation between the changes in question (14, 7). Demineralization of bone secondary to sarcoidosis has been observed rarely (22). However the sensitivity of X-ray studies in the evaluation of bone density is known to be poor since the mineral loss must be at least 30% before it can be demonstrated (15).

Study of the uptake of ^{85}Sr has recently gained an established role in the evaluation of bone accretion (6, 9). On the other hand the γ -transmission method has proved to be a highly sensitive and reliable procedure for studying bone mineral content *in vivo* (2, 36). However we have been unable to trace a study in which bone density has been measured by this method in patients with sarcoidosis.

The purpose of our work was to study bone mineral turnover in patients with sarcoidosis by measuring the uptake of ^{85}Sr in the spine and the bone mineral content using the ^{241}Am γ -ray transmission method.

MATERIAL

Twenty-one untreated sarcoid patients—14 women and 7 men—were studied. The female age range was 33-77 years (mean 48) and the male 31-74 years (mean 48). The patients had not taken hormones, vitamin D or other drugs known to affect the calcium metabolism. Other diseases which might be of significance for calcium kinetics and bone mineral balance were ruled out by chemical and laboratory tests. None of the patients

Table 1 ^{87}Sr uptake in the spine of controls and patients with sarcoidosis ^{87}Sr uptake (counts/l ml)

Controls	Patients	Significance*
Mean \pm 1 S D	Mean \pm 1 S D	<i>P</i>
Females		
13 3 990 \pm 870	14 2 820 \pm 960	0.18 <0.05
Male		
22 2 980 \pm 840	7 2 630 \pm 390	1.42 >0.05

Student's *t* test was used for the statistical analysis.

displayed clinical or roentgenological evidence of hyper trophic pulmonary osteoarthritis.

The diagnosis of sarcoidosis was based on a typical disease picture and biopsy except in two patients for whom histological verification was missing but who were found to have characteristic hilar gland enlargement, one had negative Mantoux test 100 TU and the other indocyclists. Enlarged hilar glands were encountered in 20 cases, lung parenchymal changes in 8. Four patients had characteristic skin changes and one had indocyclists. The onset of the disease was estimated in 14 cases from the subjective symptoms and in 7 on the basis of the changes seen at mass miniature X-ray. The exact time of onset is naturally uncertain, especially in the last mentioned group.

The patients were divided into three groups according to the disease picture. Group I patients with subacute active sarcoidosis of less than two years duration (14 cases: 5 men and 9 women); group II patients with subacute inactive sarcoidosis which had lasted two years at the most and who were subjectively asymptomatic and displayed only minimal roentgenographic residual (4 cases: 1 man and 3 women); group III patients with chronic sarcoidosis (3 cases: 1 man and 2 women) whose disease had lasted for three, four and ten years and in whom the diagnosis had been fixed clinically and histologically. The control group the ^{87}Sr measurement comprised 29 subjects (19 men and 10 women). The age of the men ranged from 20 to 61 years, mean 45 and that of the women from 32 to 87 years, mean 47. The measurements were made during the same period as for the patients. The indication for radiostrontium examination in these cases was low back pain, there was no X-ray or clinical evidence of local or generalized disease affecting the bone or calcium kinetics. The control group for mineral measurements has been presented earlier (1).

METHODS

The ^{87}Sr uptake in the spine of the subjects was measured using the standard method for our laboratory (1). The tracer used 50 μCi of ^{87}Sr as strontium chloride was administered 1 week before the

count. The counting apparatus consisted of a 175 \times 75 NaI(Tl) scintillation detector and an analyzer-scaler device. The radioactivity in bone was counted from the spine. Measurements were made in a skin field of 3 \times 5.5 cm. The crystal-skin distance was 10 cm. Back ground measurements were performed by covering the measuring field with a 1.7 cm thick lead shield. The measuring time was 1 min. The mean of five measurements in the Th VIII-XII region was entered as the uncorrected result. The result was corrected for the injected dose by measuring the activity of the tracer syringe before and after injection by a standard measurement geometry and comparing it with the activity of a standard dose selected by us. The stability of the measuring equipment was taken into consideration by measuring with standard geometry a certain ^{87}Sr standard while giving the injection and while taking the count, and taking into account the 65-day half-life of the ^{87}Sr radioisotope measured.

The bone mineral was measured by a modification of the γ -radiation attenuation method (20). The source of the radiation was 45 mCi ^{241}Am radioisotope γ -energy 60 kV. The forearm to be measured was fixed in a waterbath between the source and detector. At the measuring site of the bone the result recorded was the mean of four scans across the bone. The measurement was made about 1.5 cm from the distal end of the right radius (the region of cancellous bone). The mineral density (g/cm³) was calculated from the equation

$$q = \frac{K\lambda}{A} \quad \text{where}$$

$$K = 1 + \mu \exp \left(-b \sum_{i=1}^n \ln \frac{I}{I_0} \right)$$

$$\lambda = \frac{\Delta x \rho_m}{\mu_m \rho_m - \mu_w \rho_w} \sum_{i=1}^n \ln \frac{I}{I_0}$$

or the mineral linear density (g/cm) of the bone and A (0.47($\pi^2 + 1$)) is the cross-sectional area of the bone at the measuring site. The other symbols are: $a = 5.4^\circ$, $b = 4.95 \text{ cm}^2/\text{l}$ = photon fluence rate that has penetrated through water (soft tissue), I = photon fluence rate that has penetrated bone and water, μ_m = mass attenuation coefficient for bone mineral (0.39 cm^2/g), ρ_m = macroscopic density of bone mineral (2.70 g/cm^3), μ_w = mass attenuation coefficient for water (soft tissue) (0.203 cm^2/g), ρ_w = density of water (soft tissue) (1.00 g/cm^3), Δx = the distance of the points across the bone where the summation of $\ln(I/I_0)$ was made (0.10 cm), n = the number of the points from which λ was calculated.

On the assumption that the shape of the cross-section of the compact bones in the forearm is circular, mineral density index was calculated from $q = \lambda/A$ where $A = \frac{\pi}{4} (d^2 - d_0^2)$, d and d_0 are the outer and inner diameters of the bone measured from X-rays. Measurement of cortical bone was made at the point between the middle and distal thirds of the radius and ulna. The

Table II Mineral linear density and mineral density of the distal radius of the patient and controls

Density	Group	Bone mineral		Difference (%)	Significance ^a		
		Patients (mean \pm 1 S.D.)	Controls (mean \pm 1 S.D.)		t	p	
λ (g/cm)	I	14	1.45 \pm 0.28	1.30 \pm 0.29	10.0	3.47	<0.005
	II	4	1.34 \pm 0.25	1.27 \pm 0.27	5.5	0.57	>0.05
	III	3	1.12 \pm 0.30	1.18 \pm 0.28	-4.1	-1.00	>0.05
	$\eta + \delta$	1	1.37 \pm 0.29	1.28 \pm 0.27	7.0	.49	<0.025
	η	14	1.11 \pm 0.18	1.10 \pm 0.08	10.0	1.76	<0.02
	δ	7	1.34 \pm 0.08	1.64 \pm 0.17	0.0	0.00	>0.05
ρ (g/cm ²)	I	14	0.310 \pm 0.06	0.282 \pm 0.028	9.9	1.95	>0.05
	II	4	0.304 \pm 0.037	0.288 \pm 0.015	5.6	0.6	>0.05
	III	3	0.257 \pm 0.043	0.259 \pm 0.007	-0.4	-0.03	>0.05
	$\eta + \delta$	21	0.301 \pm 0.037	0.290 \pm 0.025	7.5	1.9	>0.05
	η	14	0.291 \pm 0.047	0.270 \pm 0.016	7.8	1.83	>0.05
	δ	7	0.321 \pm 0.074	0.300 \pm 0.030	7.0	0.83	>0.05

Student *t*-test and paired comparison were used for the statistical analyses.

methods used and the constants required have been presented in detail earlier (1).

The statistical analysis of the results of bone mineral measurements comparison was made with normal material (1). The control values were taken at point corresponding to the patient's age from the regression line for the right hand. The regression line was calculated in the range of 30-81 years for women and 30-87 years for men.

X-ray examination of hands and the lumbar spine was performed in all the patients and serum alkaline phosphatase and creatinine were also measured in all. Serum electrophoresis was performed in 19 patients. A total of 66 serum calcium and 63 serum inorganic phosphorus determinations were made by testing all but two of the patients at least twice. The test was performed only once in these two cases. During standardized diet containing 800 mg calcium and 1200 mg phosphorus the 4-hour urinary excretion of calcium as measured in 20 patients and phosphorus excretion in 19. The 4-hour urinary hydroxyproline excretion was studied in 18 cases while on gelatin-free diet.

RESULTS

The results for ⁸⁵Sr uptake in the spine are given in Table I. The bone mineral linear density and mineral density of the distal radius are presented in Table II. The difference $\Delta\rho$ ($\rho_{\text{pat}} - \rho_{\text{norm}}$) in the mineral density was negatively correlated with ⁸⁵Sr uptake. The correlation was statistically significant for the total material ($n = 1$, $r = -0.51$) and for women ($n = 14$, $r = -0.60$). For men too there was a tendency but it was statistically non-significant ($n = 7$, $r = -0.53$). In group I the $\Delta\rho$ was negatively related to the measurements for the duration of the disease. The corre-

lation was not statistically significant ($n = 14$, $r = 0.29$) (Fig. 1). In the diaphysis of the radius and ulna there was no statistically significant difference in the linear density and density between the controls and the patients. The per cent difference was mostly smaller than 5.

Only minor degenerative changes and positional errors in the lumbar spine were demonstrated on X-rays. Table III shows a part of the laboratory results.

DISCUSSION

Changes in the calcium metabolism in sarcoidosis established in earlier studies have been similar to those seen in vitamin D intoxication and even small doses of vitamin D have been toxic for some patients with sarcoidosis (8, 17, 15, 3). It has therefore been thought that it might be a question of an endogenous sensitivity not accurately

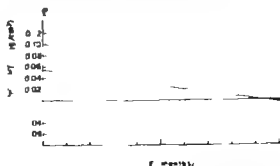


Fig. 1 Correlation between the mineral increment and the duration of the disease in the measurement in group I.

Table III Clinical and laboratory data of 21 sarcoid patients

Pat. no	Sex	Age (y)	Group	Duration from verification of disease to examination (mo)	Mean serum calcium (mmol/l)	Mean serum phosphatase (mmol/l)	Serum alkaline phosphatase (U/l)	Urinary calcium excretion (mmol/24 h)	Urinary phosphate excretion (mmol/24 h)	Tubular phosphate reabsorption (%)	Urinary hydroxyproline excretion (μmol/m ² /24 h)	Serum creatinine (μmol/l)
1	♀	42	I	3	2.40	0.83	3	4.88	26.3		170	95
2	♀	52	I	9	2.85	1.05	27	8.90	29.2	79		60
3	♀	38	I	9	2.45	1.11	20	6.95	39.8	75	130	60
4	♂	64	I	5	2.50	1.17	17	9.36	43.1	72	137	95
5	♂	31	I	2	2.50	1.08	19	6.40	36.5	85	157	100
6	♀	54	I	4	2.40	1.35	49	2.84	36.4	71	117	75
7	♀	46	I	2	2.50	1.00	20	5.74	26.0	89	104	85
8	♂	33	I	1	2.50	1.30	48	5.35	79.4	89	168	95
9	♂	4	I	5	2.55	1.25	23	4.15	70.9	95	116	85
10	♂	42	II	5	2.40	1.98	34	2.33	31.8	72	115	90
11	♂	74	III	120	2.40	1.20	79				145	130
12	♀	33	I	4	2.35	0.96	28	5.04	50.5			95
13	♂	46	I	4	2.25	0.83	27	7.60	45.4			75
14	♀	72	I	9	2.5	1.20	32	2.60	10.2	90	103	75
15	♀	60	III	36	2.50	1.18	44	2.88	6.1	96	76	80
16	♀	50	II	1	2.40	1.04	25	3.34	6.8	94	97	90
17	♀	39	I	3	2.40	1.1	41	8.25			95	95
18	♀	41	II	5	2.45	1.15	13	4.50	79.0	73	113	95
19	♀	53	III	48	2.40	1.04	20	2.48	22.1	82	97	75
20	♀	34	I	7	2.35	1.00	19	3.48	28.3	82	115	95
21	♀	35	II	12	2.40	1.33	31	3.12	27.8	87	84	65
Normal					2.25-2.75	0.81-1.45	13-49	1.3-7.3	<6.0	70-90	63-179	60-115

known to vitamin D (3 5 13 77 38). On the other hand the sensitivity to vitamin D of different organs (gastrointestinal tract bones kidneys) varies from one individual to another (10 17).

The results of the calcium balance studies performed have varied with the test arrangements e.g. the magnitude of the calcium intake (3 8 14 16 17 26 27). The disease of practically all the patients has been chronic and accompanied by hypercalcaemia (and/or hypercalcauria and renal insufficiency some of the patients have received vitamin D. These circumstances make interpretation of the results difficult and generalization impossible. The calcium kinetics of bone has been studied only in a few selected patients. The calcium turnover of bone has been either slower (23)

faster (7 16) than normal. Chronic overdosage vitamin D has led to bone mineral loss (37). Sarcoidosis could also be expected to lead to increasing porosity of bone. In spite of the great interest in the changes that occur in the calcium metabolism no studies on the bone density of sarcoid patients have been published so far.

Anttonen et al (4) reported higher than normal spinal ⁸⁵Sr uptake values in patients with sarcoidosis whose disease was chronic. In our work the uptake of ⁸⁵Sr was lower in sarcoidosis than in a normal material which suggests that the rate of bone accretion was slower than normal at the time of examination. The difference was significant for the women. The men displayed a similar tendency. However the ⁸⁵Sr uptake values for both women and men were higher in the later phase of the disease than in the patients who were examined in an early phase. The difference was not statistically significant however.

Bone mineral linear density was distinctly (approx. 10%) higher in subacute active sarcoidosis than in the controls. The difference was statistically significant. In this group the difference in mineral density (g/cm³) too was always of the same magnitude but because of greater deviation was not statistically significant. Bone density was higher in the measurements made in the early phase of the disease than in the cases with longer duration of the disease.

The results suggest that the increase in bone

mineral density occurs before the clinical manifestation of the disease since the tendency to slow decrease in bone density was demonstrable already in the first months after diagnosis of the disease.

A statistically significant negative correlation was established between the uptake of ^{85}Sr and the density of cancellous bone.

The results for patients with subacute inactive sarcoidosis were similar but less marked.

Bone density was smaller in two out of three patients with chronic sarcoidosis than in the controls. The result is not in disagreement with earlier information. Individual cases with increased radioactivity of bone have been reported but their disease was chronic and on account of hypercalcaemia led to renal insufficiency (22). In our series one patient with chronic sarcoidosis had a slightly elevated serum creatinine value. On the other hand Jackson and Deanecker (17) and Henneman et al. (14) drew attention to the rare occurrence of bone demineralization in spite of sarcoidosis lasting for years. As cortisone and its derivatives have been in common use for a long time now it is difficult to get reliable information on bone changes caused by the primary disease.

In contrast our observation that mineral density is higher than normal in cancellous bone in the initial phase of the disease is not mentioned in the literature. It seems that evaluation of bone density in sarcoidosis is extremely difficult as the results depend on establishing the duration of the disease which is often impossible. It is often not possible to estimate the onset of the disease in practice with sufficient accuracy because of the paucity of symptoms and the great number of asymptomatic cases that are detected at X-ray examination—39% in Finnish study (34).

Mineral linear density and mineral density measured from the midshaft radius and ulna did not differ significantly from the control measurements. This is explained by the considerably faster rate of mineral changes in cancellous than cortical bone (19).

Bone X-ray revealed nothing abnormal apart from minor osteochondrosis and positional errors of the lumbar spine. Serum electrophoresis showed slight elevation of α_2 - and/or β -globulin in 10 patients. The albumin fraction was elevated 55 g/l in only one of the patients (normal 33–44

g/l). In addition a slight decrease in γ -globulin was observed in one patient with subacute and a slight increase in one with chronic sarcoidosis.

One patient in group I had hypercalcaemia and hypercalciuria and another three had hypercalcaemia as well. Their uptake of ^{85}Sr was of the same magnitude as in the other patients of the same group. The hypercalcaemia and hyperphosphaturia established in 13 patients may have been caused by the fast absorption of these minerals from the intestines.

Urinary excretion of hydroxyproline has been observed to correlate well with the bone resorption rate (23–29). The 4-hour urinary hydroxyproline excretion in our study was within the normal range. The increase in bone resorption in prolonged sarcoidosis is evidently such a slow process that it is not detectable by this kind of investigation.

Elevation of alkaline phosphatase of bony origin in the serum is correlated with accelerated mineral accretion (33). No elevated values of this enzyme were observed by us which accord well with the ^{85}Sr uptake studies.

It has been shown in recent years that the effect of vitamin D on active absorption of calcium from the intestine is due to the activation of enzymes, the synthesis of a specific transport protein and the accumulation of phospholipid in the membrane of the microvilli of mucosal cells (5–11). To the best of our knowledge the connection between the abundant phospholipid formation that occurs in sarcoidosis granulomas (30) and the metabolism of intestinal phospholipids has not been studied.

A theoretical hypothesis that might be considered is that the increase in phospholipid in the intestinal mucosal cell of sarcoid patient in the phase of origin of the disease tend to lead to accelerated absorption of calcium and phosphorus. The supply of mineral to the bones then increased which in turn lead through so far unknown mechanism to the growth of bone mineral density. The possibility of latent pulmonary osteopathy cannot be ruled out. Although the osteopathy has not been described connection with sarcoidosis Yancy et al. (31) recently observed the clubbing phenomenon in 4 of 136 patients with sarcoidosis. On the other hand, clubbing may be the first and only symptom of

pulmonary hypertrophic osteopathy (4). A condition reminiscent of vitamin D intoxication which may be encountered in chronic sarcoidosis seems to cause gradual demineralization of bone. Elucidation of these points calls for further studies of sufficiently large unselected and untreated materials.

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LETTER TO THE EDITOR

WEIGHT AND IODINE CONTENT OF THE THYROID GLAND
IN JUTLAND DENMARK

Sir

No report concerning the size of the thyroid gland in Denmark has ever been published, and no figures for iodine concentration of the gland in this area are available. Small glands are known to be iodine-rich (4) and goitres to be iodine-poor. An association between iodine concentration and size of goitres has been found (1, 2) but no statistically significant correlation has been shown.

Thyroid glands from 61 females and 156 males aged 15-90 years brought to medico-legal autopsy collected by the staff of the Institute of Forensic Medicine Århus were investigated. These persons came from all parts of Jutland and most of them were healthy before the accident causing death. All fat and muscular tissue was removed before the glands were weighed and thereafter homogenized in water to a tissue concentration of about 40 mg/ml. Duplicate estimation of iodine concentration of the homoge-

nate was done in the Clinical Laboratory Århus Kommunehospital with an auto-analyser technique. Based on the first 100 duplicate estimations the S.D. was 5%.

Distribution curves for thyroid weight are given in Fig. 1. The arithmetic mean weight of the female gland was 22.9 g (median 19.3) and of the male 15.5 g (median 22.8). The weight of the gland did not vary with age but was negatively correlated to iodine concentration of the tissue both in females ($p < 0.01$, $r = -0.31$) and males ($p < 0.0025$, $r = -0.24$). A positive correlation between body weight and thyroid weight was found for males ($p < 0.0005$, $r = 0.35$).

Iodine concentration was $374 \mu\text{g/g}$ wet thyroid tissue ± 182 for females and $4.4 \mu\text{g/g} \pm 177$ (mean \pm S.D.) for males.

In West Jutland thyroid weight was lower (mean 15.8 g against 24.8 g, $p < 0.01$) by Wilcoxon's rank sum test and iodine concentration higher (mean $496 \mu\text{g/g}$ against 322 $p < 0.0025$) than in East and North Jutland statistically significant only for females. The same tendency was found for male glands. This difference between west and east+north could be due to a washing out of iodine from the soil in east+north during the last glacial period (3) or because iodine-rich run comes from the west (5) resulting in larger iodine intake through food for the people in west Jutland.

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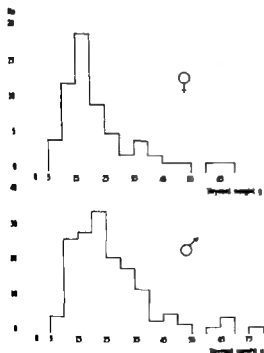


Fig. 1. Thyroid weight in males and females.

THE FUNCTIONAL PATTERN OF THE TRANSPLANTED LIVING-DONOR KIDNEY DURING THE EARLY POSTTRANSPLANT PERIOD

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Abstract. Renal function has been studied within the first 20 days after renal transplantation in 14 recipients who had received a kidney from a closely related living donor. There was a good correlation between blood flow measured with an electromagnetic flowmeter and ERBF calculated on the basis of PAH and ^{125}I -hippuran clearances. The ERPF reached 70% of the donors' bilateral preoperative ERPF during the course of ~3 hours. However the recipients' ERBF averaged only 70% of the blood flow measured in the renal artery. During the course of 70 days the recipients' glomerular filtration rate increased to 67% of the donors' bilateral preoperative value. Immediately after transplantation a change in function took place: significantly reduced filtration fraction being seen. During the course of the first 20 posttransplant days the filtration fraction approached the normal range.

Renal grafting involves implantation of a denervated organ which has been ischemic. This discontinuance of nervous regulation of renal circulation might, together with an increased sensitivity to circulating vasoconstrictor substances, affect the glomerulotubular balance (8). Mean effective renal blood flow (ERBF) has previously been studied after transplantation using ^{133}Xe (1, 7), electromagnetic flowmeters (1, 5), para-aminohippuric acid (PAH) and ^{125}I -hippuran clearances (1, 6, 10). Immediately after transplantation the relationship between mean ERBF and glomerular filtration rate (GFR) changes, resulting in a fall in the filtration fraction (FF) (1, 6). This change in hemodynamics is revealed by the isotope renogram as a change in relationship between speed of uptake and elimination and relative reduction in the speed of elimination (3).

The purpose of the present study was to investigate functional patterns of the transplanted kidney during the first three weeks after transplantation. These functional patterns were compared with functional patterns of living renal donors immediately before nephrectomy.

MATERIAL

Fourteen donors, seven men and seven women, aged 19-40, were studied immediately before renal allograft transplantation (RAT). All had normal renal function (11) (Table I).

Of the 14 recipients, aged 14-53, receiving a kidney from these donors, eight were men and six women. Each patient was studied three times, immediately after the transplantation, ~6 days, later and 7-20 days after transplantation (Tables II and III). In nine patients blood flow was measured in the renal artery just before closure of the operative incision, using an electromagnetic flowmeter (Table II).

Three of the recipients had hypertension before transplantation. In two the BP became normal immediately after transplantation (Table IV). In all recipients bilateral nephrectomy was done at the time of transplantation. In connection with the opening of the vascular anastomoses 500 mg aspirin was infused. Immunosuppression consisted of azathioprine and prednisone. Azathioprine administration began 3 days before transplantation in a dose of 3 mg/kg b.w./day. Prednisone therapy was initiated 2-3 days posttransplant with 300 mg daily. This dose was reduced during the course of 1 week to 30-40 mg/day. Azathioprine administration was continued in a dose of 3 mg/kg/day.

In three patients (nos. 81, 85, 88) moderate rejection episodes were seen 4-5 days after transplantation. It was possible to control rejection on intensification of steroid treatment. In two patients (nos. 89 and 93) severe rejection was seen which resulted in destruction of the graft. In the remaining nine patients the postoperative course was without complications.

Table I Donor kidney function before nephrectomy

All clearances are corrected to standard body surface (ml/min/1.73 m²)

Donor no.	Age (y)	Sex	C _{in}	C	C _{Cr}	C _{PAH}	FF		
							C _{in} /C _{Cr}	C _{in} /C _{PAH}	C _{in} /C _{Cr}
74	46	M	85	89	102	457	437	18.6	20.4
76	48	d	92	94	108	394	411	23.4	22.9
77	49	d	74	83	107	342	343	1.6	24.2
78	50	d	119	113	135	518	402	23.0	28.1
79	42	d	101	112	134	771	689	13.1	11.9
81	44	d	98	101	88	667	633	14.7	16.0
84	47	d	105	119	105	362	396	29.0	30.1
86	60	d	115	128	118	572	492	20.1	26.0
88	52	q	87	87	87	371	352	23.5	24.7
89	13	M	53	62	75		258		24.0
91	19	q	91	104	102		489		1.3
93	60	q	100	101	93		384		26.3
95	40	q	89	102	135		523		19.5
100	25	q	94	111	97	671	571	14.0	19.4
Average			93.1	98.3	106	513	456	20.1	22.5
S.D.			16.5	17.1	18.7	152	114	5.1	4.8
Coeff. of corr.			0.84	0.37		0.96			

METHODS

Time of ischemia was determined as the time from disconnection of ERBF in the donor kidney until ERBF had been reestablished in the recipient. Immediately after removal from the donor the kidney was cooled by perfusion with Perfudex and kept at 4°C until the vascular anastomoses were finished and circulation in the kidney was restored.

Immediately before closing the operative incision the flow in the renal artery was measured with an electromagnetic flowmeter (Medicon Microflo). Simultaneous mephthalate creatinine and I-happurin clearance (C_{in}, C_{Cr}, C_{PAH}) studies were performed 0–6 hours postoperatively in 14 recipients, and in 12 PAH clearances (C_{PAH}) were studied as well (Tables II and III). The

results from the clearance studies in 9 recipients, carried out using a constant infusion technique as earlier described (11), were compared to the results obtained in the electromagnetic flowmeter studies in the same 9 recipients (Table II). All patients received parenteral 0.88% sodium chloride infused at a rate of 100–50 ml/hour. Blood sugar was normal and there was no glucosuria. Follow-up studies were made between 7 and 730 days posttransplant (Table III). In the first and second study all recipients had ureter catheter while voiding took place at the bedside in the first case.

When comparing the donors and recipients we chose to relate graft function to the donors preoperative bilateral function (in Table I). ERBF is calculated from the C_{in} and C_{Cr}. For calculation of p-values Spearman test and Wilcoxon rank-sum test were used.

Table II Recipient kidney function after renal transplantation

Clearances are corrected to standard body surface (ml/min/1.73 m²)

Pat. no.	Age (y)	Time of ischemia (min)	EL flow meter (l)	C _{in}	C _{Cr}	ERBF		ERBF (II) / ERBF (I)	ERBF (III) / ERBF (I)
						C _{in} (II)	C _{Cr} (III)		
74	14	41	430	201	222	272	300	0.63	0.70
76	47	44	440	290	299	387	398	0.86	0.88
77	41	51	420	170	10	250	309	0.60	0.74
79	47	50	500	296	326	405	447	0.81	0.89
81	47	49	1000	322	406	470	597	0.47	0.59
	36	66	1200	546	628	713	801	0.59	0.67
	28	49	625	294	308	387	401	0.62	0.65
89	17	45	400	196	316	339	395	0.49	0.79
100	25	57	390	168	198	233	275	0.67	0.68
Average	33	51	608	277	323	372	436	0.63	0.71
S.D.		7.4	293	111	11	154	168	0.13	0.10

Table III Recipient kidney function after transplantation

Clearances are corrected to standard body surface (ml/min/1.73 m²)

Pat. no.	Age (y)	Sex	Time of ischemia (min)	Urine production after (min)	Time after RAT	Proteinuria (g/24 h)	C _{in}	C	C _C	C _{PM}	C _{DI-1-2}	FF	
												C _{in} /C _{PM}	C _{in} /C _{DI-1-2}
74	14	♀	41	1	1 h	3.7	27	26	33	201	222	13.5	11.7
					2 d	0.6	50	48	61	205	776	4.4	17.4
					9 d	0.0	40	40	76	295	41	13.9	18.6
76	46	♂	43		1 h	2.1	49	45	50	790	299	16.4	15.0
					3 d	1.1	44	42	57	265	253	16.8	16.6
					9 d	0.5	78	78	95	377	394	3.8	19.8
77	41	♂	53	2	1 h	7.5	33	33	34	170	10	19.4	16.4
					3 d	0.0	48	55	57	120	177	38.4	31.1
					9 d	0.5	65	68	87	349	330	18.6	23.4
78	45	♂	67		1 h	8.0	46	40	40	197	253	3.4	15.8
					2 d	1.2	65	48	81	304	344	1.1	19.8
					9 d	1.1	66	68	74	342	306	19.3	22.2
79	47	♂	50		1 h	3.8	39	42	75	796	326	13.1	12.9
					6 d	4.2	57	65	75	446	405	10.6	16.1
					20 d	0.0	66	77	90	429	413	15.4	18.7
81	47	♀	49	3	1 h	9.0	40	34	39	722	406	1.4	8.4
					5 d	4.0	34	34	56	173	325	19.7	10.1
					20 d	0.0	49	57	61	371	268	15.3	19.4
84	53	♂	63	5	1 h	1.9	45	47	44	209	40	21.5	17
					9 d	0.0	64	63	77	295	306	1.7	20.9
					1 h	6.6	80	59	70	556	628	14.4	9.4
86	36	♀	66	2	2 d	0.0	55	51	83	474	995	13.0	8.6
					20 d	0.9	68	76	91	598	547	11.8	13.9
					1 h	6.6	47	42	52	794	308	16.0	13.6
88	28	♀	49	1	2 d	1.7	40	43	48	298	298	14.4	14.4
					10 d	0.0	46	45	90	204	47	22.6	18.3
					1 h	5.1	28	28	38	196	316	17.0	8.9
89	17	♀	43	3	3 d	1.4	34	32	45	362	328	9.4	9.6
					1 h	8.6	42	38	41	777	329	15.6	11.5
					2 d	0.9	66	67	76	258	373	25.6	18.0
91	22	♂	48	3	9 d	1.0	88	90	93	436		20.6	
					1 h	1.8	3	31	37	28		12.6	
					2 d	0.4	12	11	17	84		1.8	
93	1	♀	57	3	1 h	1.8	3	31	37	28		12.6	
					2 d	0.4	12	11	17	84		1.8	
					9 d	0.5	65	59	106	362	312	18.0	18.9
95	14	♂	45	3	1 h	2	50	39	57		312	1.5	
					3 d	0.9	87	95	95		472	20.1	
					8 d	1.1	54	57	76	546	390	9.7	14.6
100	23	♂	57	2	1 h	3.4	34	30	34	168	798	20.3	15.1
					3 d	1.3	40	49	59	288	258	17.8	19.0
					9 d	0.5	65	59	106	362	312	18.0	18.9
Average	32		52.5		1 h	5.0	42.5	37.8	45.7	264	307	17.0	13.0
					6 d	1.7	50.0	49.2	60.5	284	338	19.7	16.4
					7-20 d	0.8	64.4	64.4	81.5	371	349	17.4	18.9
S.D.			8.3		1 h	7	13.3	8.5	13.1	107	108	1.5	8
					2-6 d	1.2	18.8	20.0	19.3	105	116	8.1	9.9
					7-20 d	1.4	13.5	14.7	15.7	115	88	4.6	8

RESULTS

The 14 donors who made up a normal study material had a mean C_{in} of 98.3 ml/min and C_{DI-1-2} of 54.6 ml/min with FF of 22.5 (11) (Table I).

Measurement, using an electromagnetic flow meter 9 recipients (Table II Fig. 1) revealed the mean blood flow to be 608 ml/min (range 350-1700) ERBF measured by C_{PM} and C_{DI-1-2} was 377 ml/min (range 223-713) and 436 ml/min

Table IV *Recipient blood pressure (mmHg) before and after transplantation*

Pat. no	Before transplantation	Days after transplantation			Antihypertensive treatment before RAT
		0-1	2-6	7-20	
74	110/70	120/80	125/80	130/80	+
76	125/80	130/70	150/70	150/70	-
77	140/110	150/120	140/110	140/100	+
78	135/60	160/80	160/90	150/90	+
79	150/115	170/90	170/110	155/95	-
81	130/80	140/90	150/90	140/95	-
84	60/110	80/90		160/90	+
86	145/90	140/70	135/85	150/90	-
88	140/90	140/90	150/80	160/100	+
89	110/60	95/40	135/75	140/90	-
91	150/90	155/70	150/90	135/80	-
93	160/100	160/100	170/100		-
95	130/80	130/100	130/95	170/90	-
100	130/70	130/80	140/85	155/75	-
Average	137/85	141/82	147/89	147/88	
S D	16/18	21/20	14/12	13/10	

(range 275-805). The relationship between flow measured by C_{PAH} and electromagnetic flowmeter was 0.63 and between flow measured by C_{125-I} and electromagnetic flowmeter it was 0.73. There was a significant positive correlation ($p < 0.001$) between flow measured with the electromagnetic flowmeter and ERBF calculated on the basis of C_{PAH} and C_{125-I} .

Three clearance studies were carried out in 14 recipients from immediately posttransplant to 20

days after operation (Table III, Fig. 2). Ischemia time was relatively constant at 52.5 ± 8.3 . Urine production took place immediately after opening of the vascular anastomoses in all cases. C_{125-I} , C_{125-I} and C_{Cr} 0-7 hours after transplantation were found to be less than half of the donors' preoperative bilateral values, viz. 45, 38 and 45% respectively. The 2-6 days posttransplant values were 50, 48 and 54% of the donors' values. In the third study GFR had increased to 63, 64 and 71% of donor values. At the time of the first two studies there was a good correlation between C_{125-I} and C_{Cr} ($p < 0.001$). In the third study C_{125-I} had increased most,

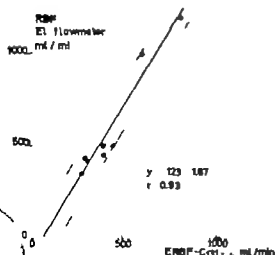


Fig. 1 ERBF calculated from ^{125}I -hippurate clearance compared to that measured by electromagnetic flowmeter.

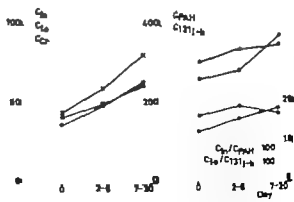


Fig. 2 Renal ^{125}I -hippurate, creatinine, PAH and ^{125}I -hippurate clearance values and filtration fractions in the three studies.

but the correlation to C_{125} was still positive ($0.025 > p > 0.01$). C_{125} and $C_{125-1-3}$ were 54 and 67% respectively of donor clearance in the first study 44 and 67% in the second and 69 and 73% in the third.

In the first two studies $C_{125-1-3}$ showed higher values than C_{PAH} , the $C_{PAH}/C_{125-1-3}$ ratio being 0.85 ± 0.09 and 0.82 ± 0.21 . In the third investigation the $C_{125}/C_{125-1-3}$ ratio was 1.05 ± 0.14 . There was a positive significant correlation between C_{125} and $C_{125-1-3}$ in the three investigations ($0.005 > p > 0.001$, $0.05 > p > 0.025$, $0.005 > p > 0.001$) and a positive non-significant correlation between the donors and the recipients clearance values. Proteinuria was 5.0, 1.7 and 0.8 g/l in the three studies. Serum albumin was constant at values of 3.59 ± 0.49 , 3.31 ± 0.53 and 3.69 ± 0.39 g/100 ml. The FF $C_{125}/C_{125-1-3}$ increased significantly ($0.025 > p > 0.01$) based on values obtained in the first and third studies (Fig. 2). In all studies FF $C_{125}/C_{125-1-3}$ was significantly lower than the donors FF value ($p < 0.001$ in the first study and $0.025 > p > 0.01$ in the third). In the first two studies the FF C_{125}/C_{PAH} showed higher values than the FF $C_{125}/C_{125-1-3}$ contrary to the proportion in the third study.

DISCUSSION

Fourteen donors with normal mean values for GFR, ERPF and FF were studied (11). At transplantation a healthy kidney was used as verified by functional studies and renal arteriograms. As has been shown previously the function of the donors remaining kidney increased compensatorily in all cases (11). In association with transplantation the circulation was interrupted for an average of 5–5.5 min.

The first study was performed immediately after transplantation of the deservated kidney in its new surroundings. Measurements of blood flow in the renal artery using an electromagnetic flowmeter revealed a higher flow value than demonstrated by C_{PAH} and $C_{125-1-3}$ performed immediately after transplantation. There was a good correlation between the results obtained with the two methods (Fig. 1). These results are not in agreement with those obtained in animal studies where almost identical values have been found with the two methods (1). Ischemic damage to tubular cells may explain our observations since extraction of PAH

and hippuran has been shown to decrease immediately after transplantation giving extraction ratios of 0.72/0.94 and 0.53/0.70 (6). According to our studies 1-hippuran extraction is not less than PAH extraction, since the $C_{125-1-3}$ value was higher than that of C_{125} at the time of the first study. Three determinations of ERPF showed very little change in values. Values within the lower range of normal were found 7–70 days posttransplant. At the time of the first two studies the ratios $C_{PAH}/C_{125-1-3}$ were 0.85 and 0.82 but in the third study a ratio of 1.05 was found. We have shown previously that C_{125} exceeds $C_{125-1-3}$ by about 13% in kidney donors (11) and by 0.5% in patients with renal diseases (12). In four patients with the nephrotic syndrome C_{PAH} was found to be 7% lower than $C_{125-1-3}$. These adverse ratios for $C_{125}/C_{125-1-3}$ might be due to a higher degree of protein-binding capacity of $C_{125-1-3}$ than of PAH. Serum albumin however did not vary in the three studies. At the time of the first study severe proteinuria was present, whereas proteinuria at the time of the two following studies was slight. The urinary content of 125 I-hippuran was independent of whether the protein present was eliminated by sedimentation or centrifugation. Thus we have no explanation for these observations of the adverse $C_{PAH}/C_{125-1-3}$ ratios but similar results have earlier been published by Kozlitz et al. when investigating renal transplants in the early postoperative course (6).

A reduced GFR revealed at determination immediately after transplantation, resulted in significant reduction of FF. During the course of the first 70 days the FF increased in patients not evidencing graft rejection. This change in FF in association with transplantation was independent of the recipients BP before transplantation (Table IV). In 13 patients normal BP was measured during the entire period of study. Previously a significant correlation between serum albumin concentration and FF has been demonstrated in patients with renal diseases (1). A relationship between serum albumin concentrations and values for FF cannot be demonstrated in the present study since serum albumin concentrations were constant. In two patients (nos. 89 and 93) whose renal grafts were destroyed because of severe acute rejection during the first 10 posttransplant days a much lower value for FF was found at the time of the second study. This change in FF has

been well demonstrated in association with a well defined ischemic period and rejection. This was also manifest in the second phase of the renogram which is an expression of the balance between rate of uptake and elimination of ^{131}I -hippuran in the kidney: the rate of uptake in these situations compensating for the rate of elimination (2, 3, 9). We have no explanation of this but intrarenal oedema affecting the glomerulus and causing a reduction in the tubular flow could be the cause (9).

Comparison between donor and recipient values for GFR showed that clearance values in the recipients reached 67% of the donors' total pre-operative value within 20 days after transplantation. The recipients' clearance increased steadily between the study periods. ERPF was found to correspond to 70% of donor values during the whole study period. There was a positive non-significant correlation between donor and recipient clearance values.

Study of a transplanted kidney from a closely related donor where ischemia time was short and well defined revealed characteristic changes in GFR and ERPF. The ERPF reached a value corresponding to 70% of the donors' bilateral pre-operative ERPF during the course of a few hours whereas the GFR reached 70% of the donors' GFR within 20 days posttransplant and thus the FF approached the normal range during this period. In spite of the relatively high values for GFR and ERPF, ERBF averaged only 73% of blood flow measured in the renal artery.

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RENAL ARTERIOVENOUS FISTULA—A CAUSE OF HEART FAILURE

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Abstract Two cases of renal arteriovenous fistula are reported in which heart failure was a major symptom. One patient had atrial fibrillation and suspected mitral insufficiency. She had observed thrill over her left flank, and a large renal arteriovenous fistula, probably congenital, was disclosed. Blood flow through the fistula was measured by dye-dilution technique and estimated to be 4.8 l/min. Cardiac output preoperatively was 6.8 l/min, after nephrectomy 4.3 l/min. Heart size diminished from 690 to 450 ml/m² BSA. The other patient had a large renal carcinoma and considerable arteriovenous shunting in the tumour. Her heart size diminished from 770 to 580 ml/m² BSA after nephrectomy and her clinical condition improved. About two months after operation she died of acute pulmonary oedema.

Renal arteriovenous fistulae, previously thought to be very uncommon, have been reported more frequently in recent years (5-7). They may cause hypertension due to ipsilateral renal ischaemia, cardiomegaly and other signs of heart failure, and macroscopic or macroscopic haematuria with or without flank pain.

We recently observed two cases of renal arteriovenous fistula in which cardiac failure was a major symptom. Special haemodynamic studies were performed in one of these patients.

CASE REPORTS

Case 1

The patient was a 54-year-old woman who had observed irregular heart rhythm for more than five years, increased weakness after exertion for about one year, and some loss of weight. She had physical signs of mitral incompetence and underwent haemodynamic investigation and left ventricular angiography. Regarded as unfit for operation, she was readmitted to her local hospital and given disableness pension. At follow-up she told that she had felt thrill over her left flank, and strong force could now be heard over her upper abdomen and left flank. Abdominal

aortography disclosed a large central arteriovenous fistula in her left kidney.

She was admitted to the Department of Urology, University Hospital, Lund, for operation. Left nephrectomy was performed. At the operation the left renal artery was found to be about 15 mm wide and tortuous, and the left renal vein was over 35 mm in diameter.

The postoperative course was uneventful. Her general condition improved, and signs of heart failure disappeared even if atrial fibrillation persisted.

Case 2

The patient was a 77-year-old woman complaining of fatigue intermittently for two months and some night incontinence. She had also observed macroscopic haematuria. She was examined at her local hospital and was found to have peripheral pitting oedema and cardiac enlargement. Abdominal aortography disclosed a large right renal tumour with considerable arteriovenous shunting.

The patient's general condition was poor and she received the suggestion of an operation very reluctantly. As the massive arteriovenous shunting in the tumour was considered an important cause of her poor condition, radical operation was, however, strongly advised. At the operation the right renal artery was found to be of about 15 mm internal diameter and the renal vein similar in size to the artery. An accidental lesion to the renal vein caused bleeding of about 7000 ml. Right nephrectomy was performed.

The general condition improved considerably after operation, but she died about two months later of acute pulmonary oedema.

METHODS

In case 1 determination of cardiac output was performed by dye-dilution technique 11 months preoperatively and 10 days postoperatively using bromocresol purple as indicator (1,3). Selective renal angiography was performed via percutaneous femoral artery catheterization (10). At the time of renal angiography, catheter was introduced also into the left renal vein, as the femoral vein. Blood flow through the renal artery was determined with Cardiac (14). Calculation of blood flow through the fistula was done according to Ekelund et al (6). At the same time cardiac



Fig 1 Selective angiography of the left kidney in case 1. Huge arteriovenous fistula in middle part of the kidney. The main renal artery has a diameter of 15 mm and the renal vein (\rightarrow) a diameter of 25 mm. Contrast filling of the ovarian vein ($\rightarrow\rightarrow$).

output was determined using the same catheters and the same indicator.

^{125}I -R(HSA (3 μCi) was used for measurement of plasma volume 4 days before and 18 days after the operation. Total glomerular filtration rate was estimated by using ^{51}Cr -EDTA with single injection technique (2) 11 days before and 3 weeks after nephrectomy. Isotope renography was performed using standard techniques, as well as I. urography.

In case 2 abdominal aortography was performed via percutaneous femoral artery catheterization. Heart size was calculated from standard chest films in antero-posterior and lateral projections.

RESULTS (Table 1)

Case 1

I.v. urography demonstrated equally sized kidneys with normal excretion of contrast medium. The kidney pelvis on the left side was deformed.

Selective angiography of the kidneys revealed a huge arteriovenous fistula in the left kidney (Fig. 1). The renal artery had a diameter of 15 mm. Rapid contrast passage occurred to the renal vein which much widened to the ovarian vein and to the inferior vena cava.

Mean blood flow through the left kidney was 3.2 l/min (normal mean value for healthy females 434 ml/min) (8). The mean flow through the fistula was calculated to be 2.8 l/min.

Isotope renography showed normal curves on both sides. At occlusion of the left renal artery during operation BP increased from 160/69 to 185/98 mmHg but returned to the original levels within a few minutes. Pulse rate did not change.

Case 2

Renal angiography showed a large malignant tumour. Contrast medium from the 12 mm wide renal artery rapidly filled the 30 mm wide renal vein.

DISCUSSION

Heart failure is one of the most important complications of arteriovenous fistulae. The degree of influence upon the heart is correlated to the size of the fistula opening (9) and inversely correlated to the distance between the heart and the fistula (10). Fistulae localized in the lower half of the body are said to be more deleterious to the heart than those

Table 1 Laboratory findings

	Pre operatively	Post operatively
<i>Case 1</i>		
Cardiac output (l/min)	7.4	6.8
Fistula flow (l/min)	2.8	0
Total blood volume (l)	4.6	3.9
Total glomerular filtration rate (ml/min)	74	60
Heart volume (ml/m ²)	690	450
<i>Case 2</i>		
Heart volume (ml/m ²)	720	540

Six months preoperatively

in the superior vena cava region (17). Renal arteriovenous fistulae are often rather large and thus together with their location in the lower half of the body and rather close to the heart, would cause a relatively high tendency to circulatory effects. This is in accordance with clinical experience.

Varela (21) gave the first report on an arteriovenous fistula in the kidney. The increase in number of renal arteriovenous fistulae reported in recent years is mainly due to the increased use of percutaneous renal biopsy but also to the increased number of renal angiographies performed (7). Among common causes of acquired fistulae are other types of trauma either blunt or sharp renal carcinoma inflammation, and atherosclerosis (15). Congenital arteriovenous fistulae in this region are uncommon they may be solitary or in any type of angiomatous malformation. Some fistulae are classified as "idiopathic". The fistula in case 1 had no certain origin and was probably congenital. Arteriovenous fistulae have a tendency to increase in size with time (13). They may remain silent for many years until they start to grow rapidly and give clinical symptoms (22).

Diagnosis of a large arteriovenous fistula is often made from the present thrill and the bruit. In other cases renal arteriovenous fistulae may be discovered incidentally in the investigation of patients with hypertension or haematuria. Urography may give a suspicion of renal arteriovenous fistula by showing filling defects calcifications or possibly delayed excretion of contrast medium. Renal angiography is the most important method for confirmation of the diagnosis and for demonstration of the anatomical relations. During angiography catheterization also of the renal vein permits performance of dye

dilution study using Cardiogreen® (8). The curve obtained may be typical of an arteriovenous fistula (short appearance time and mean transit time) (6) and thus aids in confirming the diagnosis. It may also give an estimation of fistula flow. Cardiac output measurement can easily be made at the same investigation. It is interesting to note that in case 1 the decrease in cardiac output from the two preoperative to the postoperative values is almost exactly the same as the fistula flow measured at the time of angiography. This is in accordance with the view presented by Sumner (19) that the cardiac output is equal to the sum of the normal cardiac output and the fistula flow. Compensation for the fistula flow is said to be complete for flows up to 40% of the cardiac output (12). Direct measurements of the fistula flow and of pre- and postoperative cardiac output have to our knowledge not previously been performed in patients.

Arteriovenous fistulae secondary to renal biopsy have a strong tendency to spontaneous healing within the first few months after induction (4, 7). In other cases surgical treatment should be resorted to in most cases nephrectomy is performed. In recent years several cases have been reported in which the fistula could be repaired with salvage of the kidney (5, 18, 20).

In the present cases nephrectomy had to be performed. In case 1 the central location of the fistula made conservative surgery impracticable. Ligation of the feeding artery proximal to the fistula, Hunterian ligation, might have been possible. In other parts of the body Hunterian ligation is a poor treatment for arteriovenous fistula (13) as collaterals keep the fistula going. Collaterals may develop even in the kidney (1), which would make simple central arterial ligation an unsatisfactory technique also in the kidney.

Of common complications to arteriovenous fistulae heart failure was the only apparent symptom in the present cases. The suspected mitral valve insufficiency in case 1 was probably the effect of cardiac dilatation. Cardiac changes as well as asexual morphological changes are not always reversible after repair of fistula. This is illustrated by the outcome in case 1. Even in case 1 although clinical symptoms of cardiac failure have disappeared and there is a considerable improvement of the subjective state atrial fibrillation remains.

In the treatment of large arteriovenous fistulae it should be observed that blood volume is often larger

than normal. When the fistula is closed peripheral resistance increases abruptly and the enlarged blood volume may lead to acute pulmonary oedema (3, 11).

Continuous monitoring of central venous pressure and of arterial pressure during operation is recommended and administration of fluids should be done with care. The patient should be observed for several days postoperatively and venesection of 500–1000 ml blood may be necessary. In patient 1 pulse rate did not decrease when the fistula was occluded but there was a temporary rise in arterial pressure.

The glomerular filtration rate after operation was in case 1 reduced relatively little compared to the operative value. This could indicate that the function of the left kidney was reduced. On the other hand the renal size was equal on both sides and the contrast excretion at urography and the curves registered at isotope renography were essentially normal. These findings indicate that renal ischaemia was not present which accords with the absence of arterial hypertension. The separate function of each kidney in patient 1 calculated from renography curves and from examination with a scintillation camera (Larsson et al. unpublished data) showed the function of the left kidney to be only slightly to moderately reduced as compared to the right kidney. The satisfactory preservation of glomerular filtration rate postoperatively accordingly most probably indicates good compensatory hypertrophy of the remaining kidney.

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RAPID EVALUATION OF CREATININE CLEARANCE

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Abstract Endogenous creatinine clearance, serum creatinine and urinary creatinine excretion have been determined in 368 patients hospitalized with various medical diseases and without known renal disease. The patients were divided into 10-year age groups and the results were analyzed for age and sex differences. The average creatinine clearance in males showed steady decline from 110 to 34 ml/min/1.73 m² from the age group 20-29 to the age group 90-99 years. The mean serum creatinine levels did not show any significant variations with age, but urinary creatinine (mg/kg b.wt./24 h) decreased from 23.8 in the age group 20-29 to 9.4 in the age group 90-99 years. In females creatinine clearance declined from 93 to 39 ml/min/1.73 m² serum creatinine was without significant change and urinary creatinine (mg/kg b.wt./24 h) decreased from 19.7 in the age group 20-29 to 8.4 in the age group 90-99 years. The finding of considerably decreased creatinine clearance values without elevation of serum creatinine values in the elderly is probably explained by great reduction with age in lean body mass and thereby in creatinine production. In a group of 106 patients with serum creatinine values between 1.4 and 5.0 mg/100 ml the urinary creatinine (mg/kg b.wt./24 h) was not significantly different from that of patients without renal disease. A risk of overestimation of glomerular filtration rate seems to exist if serum creatinine is used as the only parameter of renal function in these patients and might lead to intoxication with drugs which are mainly excreted by the kidney. The authors suggest nomogram for rapid evaluation of creatinine clearance in ml/min.

Estimation of serum creatinine has been widely adopted as a screening method for renal function in clinical work (6, 7, 8, 19). A considerable decrease in glomerular filtration rate (GFR) in the elderly was demonstrated by Lewis and Alving (15) and later confirmed by others (4, 17, 21). However serum creatinine determinations in larger groups of healthy persons have shown no significant change with age (5, 13, 14). The findings of decreased GFR and unaltered serum creatinine

values in the elderly probably indicates a decrease in endogenous creatinine production with age and a fall in urinary creatinine per kg b.wt. in the elderly has also been demonstrated by Ahlert et al. (1) and by Bahrs et al. (3).

We have studied urinary creatinine, serum creatinine and endogenous creatinine clearance in different age groups. From these values we suggest a rapid nomographic evaluation of the GFR taking the influence of sex, age, body weight and serum creatinine into consideration.

MATERIAL AND METHODS

The material includes patients who had been admitted to three medical departments in three hospitals in Copenhagen. The composition of the material is seen from Table I. The main diagnosis was essential hypertension in 114 patients and chronic nephropathy in 106. Most of the elderly suffered from arteriosclerotic and cerebral disorders.

In each sex group all the patients were divided into 10-year age groups. Body weight (kg), height (cm), serum creatinine according to the method of Folin (mg/100 ml) and the 4-hour endogenous creatinine clearance (ml/min) were determined in all patients. Many of the elderly had with different indications catheter & drainage which ensured that the urine was collected quantitatively. Urinary creatinine was determined in 3 consecutive 4-hour samples and the mean value was used. Only variations in 4-hour creatinine excretion less than 25% were accepted. Body surface according to the method of du Bois and urinary creatinine (mg/kg/24 h) were calculated.

RESULTS

Table I shows the results from 149 men and 219 women with serum creatinine values ≤ 1.4 mg/100 ml and from 51 men and 55 women with serum creatinine values between 1.5 and 5.0 mg/100 ml.

Table I Results from 149 males and 219 females with serum creatinine ≤ 4 mg/100 ml and from 51 males and 55 females with elevated serum creatinine values between 1.5 and 5.0 mg/100 ml

Age (y)	B wt. (kg)	Clearance		Serum creat. (mg/ 100 ml)	Urinary creat.			n ^a	Urinary creat. (mg/kg/ 24 h)
		ml/min	per 1.73 m ²		mg/ 24 h	mg/kg/ 24 h	mg/kg/ min \times 100		
Males									
20-29	12	68.4	117 \pm 23	110	0.99 \pm 0.18	1 635 \pm 137	23.8 \pm 2.3	1.65	0
30-39	10	70.9	98 \pm 39	97	1.14 \pm 0.22	1 520 \pm 130	21.9 \pm 1.5	1.52	4
40-49	32	77.5	98 \pm 22	88	1.10 \pm 0.20	1 544 \pm 421	19.7 \pm 3.2	1.37	7
50-59	37	75.7	88 \pm 21	81	1.16 \pm 0.17	1 445 \pm 252	19.3 \pm 2.9	1.34	14
60-69	23	73.4	76 \pm 22	72	1.15 \pm 0.14	1 252 \pm 364	16.9 \pm 2.9	1.17	13
70-79	18	69.5	64 \pm 15	64	1.03 \pm 0.22	919 \pm 13	14.2 \pm 3.0	0.99	10
80-89	12	56.3	45 \pm 15	47	1.06 \pm 0.25	651 \pm 238	11.7 \pm 4.0	0.81	1
90-99	5	67.6	35 \pm 9	34	1.20 \pm 0.16	612 \pm 188	9.4 \pm 3.2	0.65	2
Total	149								51
Females									
20-29	37	58.1	91 \pm 19	95	0.89 \pm 0.17	1 135 \pm 224	19.7 \pm 3.9	1.37	0
30-39	14	60.9	96 \pm 25	103	0.91 \pm 0.17	1 218 \pm 191	20.4 \pm 3.9	1.42	4
40-49	48	60.1	76 \pm 26	81	1.00 \pm 0.24	1 056 \pm 256	17.6 \pm 3.9	1.22	10
50-59	34	67.8	74 \pm 24	74	0.99 \pm 0.26	989 \pm 246	14.9 \pm 3.6	1.04	11
60-69	23	65.4	60 \pm 15	63	0.97 \pm 0.17	871 \pm 283	12.9 \pm 2.6	0.90	13
70-79	27	58.3	49 \pm 12	54	1.02 \pm 0.23	685 \pm 184	11.8 \pm 2.2	0.82	1
80-89	11	55.3	41 \pm 14	46	1.05 \pm 0.22	578 \pm 154	10.7 \pm 2.5	0.74	4
90-99	9	52.4	34 \pm 8	39	0.91 \pm 0.12	433 \pm 113	8.4 \pm 1.4	0.58	1
Total	219								53

Patients with elevated serum creatinine.

Evaluation of endogenous creatinine clearance (ml/min) -

urinary creatinine (mg/kg/min) \times weight (kg) $\times 100$
serum creatinine (mg/100 ml)

In the males the mean b wt. increased from the age of 20 to the age of 50 and then a gradual decrease was found. The mean height in the age group 20-29 years was 178.7 cm and decreased gradually to 168.4 in the age group 90-99 years. Changes in the calculated body surface showed variations from 1.84 m² in the age group 20-29 years to 1.91 m² in the age group 40-49 years. mean body surface then decreased to 1.63 m² in the patients aged 80-89 years.

Endogenous creatinine clearance in ml/min and corrected to a body surface of 1.73 m² showed constantly decreasing mean values with a gradual fall of about 10 ml/min/10 years. The mean serum creatinine values showed only small variations with age and after the age of 30 years no significant differences were found. Total urinary creatinine values showed a moderate decrease from the age group 20-29 to the age group 50-59 and thereafter a more pronounced fall was observed. The mean urinary creatinine value in mg/kg b wt./24 h showed a constant decrease from the youngest

to the oldest age groups and the mean value decreased from 23.8 to 9.4 mg/kg/24 h. No significant difference in the mean values of urinary creatinine/kg b wt./24 h was found between patients with serum creatinine values of 1.5-5.0 mg/100 ml and those in corresponding age groups with serum creatinine values below 1.5 mg/100 ml.

In general all parameters for the females show values lower than the corresponding values for males. The decrease with age in clearance in total urinary creatinine and in urinary creatinine/kg b wt./24 h was of the same degree as in the groups of males. Also in the females with serum creatinine between 1.5 and 5.0 mg/100 ml the mean urinary creatinine values did not differ significantly from those for the patients with serum creatinine ≤ 1.4 mg/100 ml.

DISCUSSION

Variations in renal clearance with age have been recognized for many years (4, 15, 17, 18). In pre-

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DOSE REGIMEN OF KANAMYCIN AND GENTAMICIN

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Abstract The half-life of kanamycin and gentamicin have been determined in 74 and 49 patients, respectively. In patients with normal serum creatinine values the kanamycin $T_{1/2}$ varied from 90 to 538 min and the gentamicin $T_{1/2}$ from 40 to 370 min and in both cases an inversely proportional relationship with creatinine clearance was found. The half-lives of both drugs increased with advancing age reflecting the age-dependent decrease in endogenous creatinine clearance which takes place with unaltered serum creatinine values. This finding invalidates many calculations of drug half-lives based solely upon serum creatinine values. Using previously described nomogram it is possible to evaluate a patient's creatinine clearance if the age, sex, weight and serum creatinine are known. Calculations of kanamycin and gentamicin half-lives based upon evaluation of creatinine clearance from the nomogram are compared with the measured half-lives. Dose regimens for kanamycin and gentamicin are suggested.

Kanamycin and gentamicin belong to the aminoglycoside group of antibiotics which is characterized by a narrow range between therapeutic effectiveness and pharmacological toxicity. Neither of these drugs are metabolized in the body but eliminated unchanged by the kidneys mainly by glomerular filtration. An evaluation of the renal function must therefore be the basis of dose regimens for these drugs. Some of the dose schedules given in the literature relate the excretion of kanamycin and gentamicin to endogenous creatinine clearance but this is a rather difficult parameter to obtain quickly in clinical practice (6, 9, 11).

In some recent papers concerning schedules of treatment with kanamycin and gentamicin serum creatinine has been used as the only parameter of renal function and it has been suggested that the half-lives in hours of these drugs could be estimated by multiplying the serum creatinine concentration in mg/100 ml by three for kanamycin and by four

for gentamicin (3). However serum creatinine is of little value in predicting creatinine clearance unless it is taken into consideration that urinary creatinine excretion varies considerably with the sex, age and weight of the patient (4, 7).

We have previously described a rapid bedside method of making an approximate estimation of the creatinine clearance based upon the weight and the serum creatinine of the patient and upon mean values of urinary creatinine excretion in different age and sex groups (7, 11). In the present study this method has been used to calculate the half-lives of kanamycin and gentamicin and the calculated half-lives have been compared to measured half-lives. Practical dose regimens for the two drugs are suggested.

MATERIAL AND METHODS

Kanamycin

The material comprised 74 patients, 35 men (20-97 years) and 39 women (70-90 years). Sixty seven of the patients suffered from functional and arteriosclerotic disorders without known renal diseases and all had serum creatinine values below 1.5 mg/100 ml. Seven of the patients had nephropathy of various origin with slightly elevated creatinine values ranging from 1.5 to 3 mg/100 ml.

Serum and urinary creatinine and 7^h hours endogenous creatinine clearance were estimated by standard procedures (Technicon AutoAnalyzer). Kanamycin half-life was determined by measuring the serum kanamycin concentration four times at varying intervals beginning 2 hours after an i.v. injection of 500 mg kanamycin sulphate. The kanamycin concentrations were determined by the agar cup method using strains of *Bacillus subtilis*. In 22 patients the kanamycin determinations were carried out twice on different days on the same serum samples. The standard deviation of the differences between double determinations of half-lives is 16.7% of the mean half-life. To evaluate the intra-individual variations in kanamycin half-life repeated de-

Table 1 Measured kanamycin $T_{1/2}$ compared to $T_{1/2}$ calculated from serum creatinine and V_d calculated from estimated creatinine clearance values and distribution volume in three age groups

Age (y)	20-50	51-70	>70
No. of pts.	13	1	33
Serum creatinine (mg/100 ml)	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Calculated $T_{1/2}$ from serum creatinine (min)	176 ± 21	171 ± 41	200 ± 67
Measured $T_{1/2}$ (min)	107 ± 7	149 ± 49	130 ± 154
Distribution volume (% of b.w.t.)	16.7 ± 3.0	16.0 ± 6.9	13.1 ± 6.1
Calculated $T_{1/2}$ from nomogram and for mean (min)	117 ± 20	164 ± 35	136 ± 140

$$T_{1/2} (\text{min}) = \text{serum creatinine (mg/100 ml)} \times 180$$

$$T_{1/2} (\text{min}) = \frac{V_d \times \text{weight (g)} \times \log_e}{0.60 \times \text{clearance}}$$

terminations were carried out in 10 patients at interval of seven days. The values of the two half-life determinations did not differ significantly (mean values 300 ± 122 and 282 ± 107 min, $p > 0.5$). Kanamycin half-life was also determined following i.v. injection of 500 mg kanamycin sulphate in 19 of the patients. These half-life determinations did not differ significantly (mean value 268 ± 52 min) from the half-lives determined after i.m. injection (mean value 296 ± 71 min, $p > 0.1$). The apparent distribution volume of kanamycin was calculated from the extrapolated kanamycin concentration at the time of injection and the total amount of the drug administered. An approximate estimate of the creatinine clearance was carried out in all the patients, using a previously constructed nomogram (7). Using these values of creatinine clearance, kanamycin half-life was calculated from the formula

$$T_{1/2} = \frac{V_d \times \log_e}{\text{clearance}} = \frac{V_d \times 0.693}{\text{clearance}}$$

V_d is the apparent volume of distribution and is the total plasma clearance of kanamycin. According to Orme and Cutler (9) the total plasma clearance of kanamycin is about 60% of endogenous creatinine clearance for the whole range of renal function.

Gentamicin

The material comprised 49 patients, 14 men (20-64 years) and 35 women (18-94 years). All the patients suffered from functional and arteriosclerotic disorders without known renal diseases and all had serum creatinine values below 1.5 mg/100 ml.

Gentamicin half-life was determined like kanamycin half-life but after an i.m. injection of 80 mg gentamicin sulphate. The gentamicin concentrations were determined using a strain of *Staphylococcus aureus*. The variation of the gentamicin determination and the interindividual differences of repeated gentamicin half

life determinations were of the same order of magnitude as for kanamycin. Gentamicin half-life was calculated from the above mentioned nomogram and formula using a gentamicin clearance value of 90% of the endogenous creatinine clearance according to the study of Bergan et al. (1) for all values of creatinine clearance.

The data have been subjected to statistical analysis and a Student's *t*-test applied to a paired comparison.

RESULTS

Kanamycin

The measured kanamycin half-lives varied between 75 and 800 min and the endogenous creatinine clearances between 170 and 13 ml/min. An inversely proportional correlation between these two parameters was found. In the 67 patients with normal serum creatinine values, kanamycin half-lives varied between 90 and 538 min and endogenous creatinine clearances between 111 and 77 ml/min.

Table 1 shows the values from the 67 patients with normal serum creatinine values. The patients were divided into three age groups. The mean values of serum creatinine were practically identical in the three age groups, but a considerable decrease in creatinine clearance with age was noted with a mean value of 94 ± 17 ml/min in the youngest age group and values of 75 ± 70 ml/min and 43 ± 1 ml/min in the middle and old age groups respectively.

Kanamycin half-lives (min) were calculated by multiplying serum creatinine (mg/100 ml) by 180 as suggested by Cutler and Orme (3). In patients aged 20-50 years the actual measured kanamycin half-life was shorter than the half-life calculated by this procedure ($p < 0.001$). No significant difference was found between the measured and the calculated kanamycin half-lives in the age group 50-70 years ($p > 0.05$) but in the older patients the actual measured kanamycin half-life was considerably longer than the half-lives calculated from the serum creatinine values ($p < 0.001$). The distribution volume (% of b.w.t.) was greater in the elderly ($p < 0.001$) compared with the other two age groups. Mean values of kanamycin half-lives calculated from the above mentioned formula (where V_d is the mean value of the distribution volume of kanamycin in the three age groups and where creatinine clearance is calculated from the nomogram in every patient) are shown in the last part of the Table. It is seen that kanamycin half-life calculated in this way does not differ significantly from the actually measured half-lives ($p > 0.05$).

Table 1L Measured gentamicin $T_{1/2}$ compared to $T_{1/2}$ calculated from serum creatinine and to $T_{1/2}$ calculated from estimated creatinine clearance values and distribution volume in three age groups

Age (y)	20-50	51-70	>70
No. of pts.	12	12	5
Serum creatinine (mg/100 ml)	1.0±0.1	1.1±0.2	1.0±0.2
Calculated $T_{1/2}$ from serum creatinine* (min)	14±22	26±55	43±44
Measured $T_{1/2}$ (min)	93±76	120±25	216±60
Distribution volume (% of b.wt.)	15.±7.8	16.2±5.8	4.±5.9
Calculated $T_{1/2}$ from nomogram and formula (min)	91±13	144±33	40±57

$$T_{1/2}(\text{min}) = \text{serum creatinine (mg/100 ml)} \times 40$$

$$\div \text{weight (g)} \times \log 2$$

$$T_{1/2}(\text{min}) = \frac{0.90 \text{ clearance}}{0.90 \text{ clearance}}$$

Gentamicin

The measured gentamicin half-lives varied between 40 and 370 min and creatinine clearance between 1.5 and 111 ml/min. An inversely proportional relationship was also found between these two parameters.

Table 1I shows the values from the 49 patients examined. No significant difference was found between serum creatinine values in the three age groups whereas creatinine clearance decreased with age in a similar way as in the kanamycin material.

Gentamicin half-life was calculated by multiplying serum creatinine (mg/100 ml) by 40 as suggested by McHeary et al. (8) and Cutler et al. (3). When these calculated half-life values were correlated to the actually measured half-lives, no difference was found in the elderly patients ($p>0.05$) but in the two younger age groups the calculated half-lives were considerably longer than the measured ($p<0.001$). The distribution volume (% of b.wt.) showed higher values in the elderly ($p<0.001$). Gentamicin half-lives were calculated from the above mentioned formula and nomogram and no significant differences from the actual half-lives were found ($p>0.05$).

DISCUSSION

Our findings of inversely proportional relationship between kanamycin and gentamicin half-lives and

endogenous creatinine clearance are in agreement with previous studies (1, 5, 6). Dose schedules of these drugs must therefore be based upon appropriate estimations of renal function. Creatinine clearance or other methods for the estimation of glomerular filtration rate are usually not available in acute clinical practice. Cutler and Orme (3) therefore suggested that kanamycin half-life (min) could be estimated by multiplying serum creatinine (mg/100 ml) by 180. It has also been suggested that the half-life of gentamicin (min) could be calculated by multiplying serum creatinine (mg/100 ml) by 40 (1).

In a previous study of 479 patients we have demonstrated that urinary creatinine excretion and creatinine clearance decrease with advancing age without any significant changes in serum creatinine (7). This implies that the above mentioned calculations of half-lives suggested by Cutler and Orme with the use of serum creatinine possibly cannot be applied with satisfactory results in all age groups. Our findings of considerable age-dependent variations in measured half-lives of kanamycin and gentamicin in spite of identical serum creatinine values in the different age groups support this assumption.

Based upon sex, weight and age-dependent variations in urinary creatinine excretion we have constructed a nomogram from which it is possible to calculate the endogenous creatinine clearance with a precision of 15% (7, 11). Our nomogram has been found valid for all persons with normal serum creatinine values and also for patients with moderately increased serum creatinine values as the creatinine excretion in these patients follows the same pattern as normal persons. In severe uremia urinary creatinine excretion/kg b.wt. is decreased causing tendencies to an overestimation of the renal function when using the nomogram. The apparent distribution volume for both drugs was found to be increased in the elderly patients in accordance with several previous studies (5, 6).

If the information from the nomogram and the distribution volume are used, the general formula relating the half-life of drug to its clearance and the following two equations are obtained:

$$\text{kanamycin } T_{1/2}(\text{min}) = \frac{1 \times \text{b.wt. (g)} \times 0.693}{0.60 \times \text{clearance}}$$

$$\text{gentamicin } T_{1/2}(\text{min}) = \frac{1 \times \text{b.wt. (g)} \times 0.693}{0.90 \times \text{clearance}}$$

where $\Pi 60$ and $\Pi 90$ are the relationships between the clearance of the drug and the endogenous creatinine clearance.

In the present study of 13 patients we have shown good correlation between the calculated and measured half-lives of kanamycin and gentamicin in patients with normal serum creatinine values. The calculations consider the age-dependent change in renal function. The same accordance was seen in the seven patients with slightly elevated serum creatinine values.

When prescribing each of the two drugs it has been recommended to give the doses at an interval of three half-lives (10). The usual doses proposed are 7 mg kanamycin and 1-2 mg gentamicin/kg b.wt. These dose values and the above outlined interval should permit adequate antimicrobial therapy without dangerous accumulation of the drugs.

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DRUG INDUCED MALABSORPTION OF VITAMIN B₁₂VII Malabsorption of B₁₂ during Treatment with Potassium Citrate

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Abstract. Twelve of 40 heart patients on medication with potassium citrate had slightly low results (5-9.5%) in the Schilling test. Administration of calcium at the same time did not prevent this harmful effect. The mechanism of the disturbance is more likely to involve slight acidification of the contents of the ileum than binding of the Ca⁺⁺ ion by the citrate. The disturbance in the absorption of B₁₂ during medication with potassium citrate is usually so slight that it can cause deficiency of B₁₂ only in rare cases. This finding is more important as a transient cause of a low Schilling test.

The treatment of heart patients with slow-release tablets of potassium chloride has been shown to cause malabsorption of vitamin B₁₂ in 30% of cases (3). The mechanism involved is the acidification of the contents of the intestine. Potassium citrate is a weaker acid salt than potassium chloride and hence its effect on the absorption of vitamin B₁₂ should be milder.

The present paper reports observations on the effect of potassium citrate on the absorption of vitamin B₁₂.

MATERIAL AND METHODS

The absorption of vitamin B₁₂ was studied in 50 male heart patients in a medical ward. Patients with pernicious anaemia, fish tapeworm and other known causes of B₁₂ malabsorption were excluded. Potassium citrate Kalamix solution (Star Ltd, Tampere) 30 ml three times daily containing 10.8 g of potassium citrate (97 mEq) in 100 ml, was administered to 40 patients. To study the possibility that the binding of calcium ion by citrate might be the mechanism causing malabsorption of B₁₂, 8 g of Ca⁺⁺ (100 mEq) in the form of calcium gluconate (4 tablets Calcium Sandoz forte, Sandoz A.G. Basel) was given to the remaining 10 patients together with the Kalamix (30 ml daily).

A Schilling test with potassium was performed before treatment and on the fourth day afterwards using 1 µg ⁵⁷CoB₁₂ as test dose. Normal values in our

laboratory are >10% in 4 hours, and pathological values <5% whereas values between 5 and 10% may be considered slightly abnormal.

Measurements of the pH in the small intestine were obtained from 8 patients during potassium medication using radiotelemetric equipment (Telefunken AG Ulm, GFR). The accuracy of the method is 0.5 pH unit.

RESULTS

Schilling tests

The Schilling test results for the group of 40 patients before and during medication with potassium citrate are shown in Fig. 1. The value was slightly abnormal (actually 5.5-9.5%) in 1 patient (30%) and normal in 28 (70%). In 7 patients (17%) the Schilling test value decreased to half of the original value or below the average being 13.3% (without potassium) and 5.1% (with potassium).

Ileal pH

The ileal pH was measured in eight patients. The values observed were 6.2 in one, 6.5 in three, 7.0 in one and 7.5 or above in three patients. The pH of the Kalamix® mixture was 5.7.

Effect of calcium

In three (30%) of the patients who received calcium gluconate in addition to the potassium citrate the Schilling test value decreased to half of the original value or below, in one patient to as low as 1%.

DISCUSSION

An impaired absorption of vitamin B₁₂ was observed in 30% of our patients during medication with a solution of potassium citrate. The overall incidence of B₁₂ malabsorption was thus

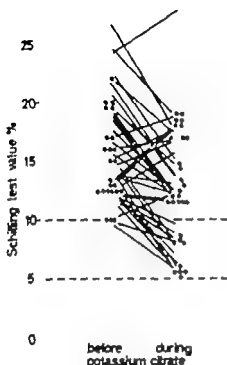


Fig 1 Schilling test values in 40 heart patients before and during medication with potassium citrate

just the same as observed earlier during potassium chloride medication (4). However while the Schilling test value was below 5% in 12% of the patients receiving potassium chloride similar values were observed in only one of 50 patients receiving potassium citrate.

In vitro experiments (1) have shown that the intrinsic factor effect on the uptake of B_{12} by ileal homogenate is dependent on the presence of iron and on the pH of the environment. The Fe^{2+} ion in the potassium citrate might bind the

calcium rendering it insoluble and thus impair the absorption of B_{12} . An equivalent dose of calcium to bind the citrate ions did not, however reduce the harmful effect of the potassium citrate. Thus it is unlikely that potassium citrate would impair the absorption of B_{12} through the binding of a calcium ion. As the pH of the potassium citrate mixture is closer to neutral than that of a 1% KCl solution (5.7 compared with 5.1) no very low pH values for ileal contents were expected. The low Schilling test values during KCl medication were connected with pH values below 6 (3). The pH values observed during potassium citrate medication are consistent with the Schilling test values and with the pH of the mixture; both the effect on ileal pH and the absorption of B_{12} are slight.

Impaired absorption of B_{12} during treatment with potassium citrate is rather frequent, but it is so mild that it can be expected to cause deficiency of B_{12} only in exceptional cases namely those already on the borderline of B_{12} deficiency for other reasons. This finding is more important as a transient cause of low values when interpreting the results of the Schilling test.

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SYSTEMIC LUPUS ERYTHEMATOSUS

Incidence and Manifestations During 14 Years in a Swedish Province

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Abstract Forty-one definite and 25 suspected cases of SLE, 66 cases in all, have been investigated at the County Hospital, Halmstad in 1957-71. The occurrence of SLE and related diseases was studied, as well as the most common clinical manifestations of SLE. The population in the area of uptake was 95 127 in 1957 and 111 707 in 1971. The incidence during 1957-64 was 17.6/year/1 mill inhabitants for definite SLE and 16.2 for suspected SLE. During 1964-71 the incidence of definite SLE was 29.7 and of suspected SLE 12.2/year/1 mill inhabitants. The increasing incidence of definite SLE is probably due to improved laboratory diagnoses, but the possibility that SLE may have become more common cannot be disregarded.

SLE is an autoimmune disease which predominantly affects young and middle-aged women and in which a wide variety of organic manifestations occur concomitantly or at different points of time. The course of the disease varies considerably: it is often chronic with numerous remissions and exacerbations, and may vary from at the one extreme spontaneous clinical recovery to at the other fulminant disease with a high mortality often due to renal failure. The immune reactions of the body are altered considerably. There are several points of evidence suggesting that the altered immune reactions are genetically determined but in addition to genetic predisposition external trigger factors of various kinds seem to be necessary. The possible etiological significance of viral infections in addition to genetic disposition has been much discussed in recent years since virus-like particles have been found in the endothelial cells of the renal vessels, the leucocytes and other organs of patients with SLE. Animal experiments and investigations of naturally

occurring autoimmune SLE-like diseases in for example NZB mice which are also virus carriers support the virus theory. The etiology of SLE is however not known and it is probable that several factors are of importance for the occurrence of the manifest disease.

Several authors have demonstrated familial aggregation of SLE and similar autoimmune conditions. Leonhardt (13) demonstrated an increased occurrence of an increase in polyclonal γ -globulin, high ANF titres and rheumatic factor in lupus patients and their otherwise normal relatives, probably indicating an altered immunological response to endogenic and exogenic stimuli of various kinds. One finds an increased occurrence of numerous humoral antibodies. Complement-binding antigen-antibody complexes on the other hand often cause tissue damage. Lupus nephritis and vasculitis are often fatal immune complex diseases. In addition there is the altered cell-mediated immune reaction which is probably of considerable pathogenic importance in SLE.

In addition to the typical clinical symptoms the diagnosis requires various positive laboratory tests such as demonstration of increase in polyclonal γ -globulin of autoantibodies of which the most important are antinuclear factors and of the LE cell phenomenon. A further confirmation of the diagnosis can be obtained by recent immunofluorescent techniques and electron microscopic investigations.

At this hospital we have studied SLE during the years 1957-71 with regard to its incidence and various manifestations and to its relation to other collagen and autoimmune diseases.

MATERIAL AND METHODS

The population of the area served by the County Hospital was 95 177 in 1957 and 111 707 in 1971. The study was commenced on 1st July 1957 and ceased on 30th June 1971. From mid-1957 to mid-1964 all patients were diagnosed in the Department of Medicine from mid-1964 to mid-1971 both in the Department of Medicine and the Department of Infectious Diseases. When calculating the incidence the patients resident outside the area of uptake have been omitted.

We have mainly applied the diagnostic criteria for SLE presented by Leonhardt (13) and have used Dubof criteria (4) for selection of SLE patients. In addition to the typical clinical signs, numerous positive laboratory findings—such as demonstration of increase in γ -globulin autoantibodies, above 11 antinuclear factors and of LE cells—were required for diagnosis. Clinical, patho-anatomical and histological biopsy and autopsy findings supported the diagnosis. It did also successful treatment with corticosteroids and cytostatics.

Mainly in accordance with Leonhardt criteria, SLE patients were divided into definite ($n=41$) and suspected ($n=25$) cases. Patients with typical clinical symptoms and typical patho-anatomical findings, patients with positive LE cells, butterfly exanthema and certain other organic manifestations typical of SLE and patients with either positive LE cells, high ANF titres or butterfly exanthema and typical clinical symptoms were allotted to group A definite SLE. If one or two did not fulfil all these criteria but had typical findings suggestive of SLE were allotted to group B suspected SLE.

Laboratory methods

γ -globulin in exudates were performed by means of paper electrophoresis in the Laboratory of Clinical Chemistry at this hospital. The upper limit for the normal value was placed at 1.45 g/100 ml. In recent years certain immunoelectrophoretic investigations were performed in the Department of Clinical Chemistry, Malmö General Hospital and in the Department of Clinical Chemistry Sahlgren Hospital Gothenburg. ANF titres determined in three laboratories: Malmö General Hospital Sahlgren Hospital Gothenburg, and the Bacteriological Laboratory Stockholm. The titres from different laboratories were not directly comparable.

LE cells were mainly investigated by the Zinkham-Conley method in the Laboratory of Clinical Chemistry at this hospital. In addition to the presence of typical LE cells in one and especially several samples, we have also considered the presence of round haematoid bodies of homogeneous type and rosettes since according to the extensive investigations of Dubof (4) the presence of round haematoid bodies and rosettes in several samples is strongly suggestive of SLE.

Anemia was defined according to Leonhardt criteria, Hb < 11 g/100 ml for men and < 10 g/100 ml for women. Leucocyte values below 3000/mm³ were considered as suggestive of leucopenia and fewer

than 100 000 platelets/mm³ of thrombocytopenia, the lower platelet value than in many other materials.

Liver cytology samples were in most cases investigated by Professor N. Söderström, Department of Medicine, Lund. Other biopsy material was mainly investigated in the Institution of Pathological Anatomy, Malmö, where also occasional autopsies were performed.

Pathological urinary sediments with 5–10 red cells/high-power field in several tests and/or proteinuria in several samples in the absence of concomitant infection were considered suggestive of lupus nephropathy. Isolated serum complement investigations were performed in the Department of Clinical Chemistry, Malmö General Hospital and the Bacteriological Laboratory Sahlgren Hospital Gothenburg. The plasma half-life (PT_{1/2}) for INH was determined in the Department of Clinical Chemistry, Karolinska Hospital, Stockholm.

RESULTS

Study of the case report revealed several diseases which might cause diagnostic difficulties with regard to SLE (Fig. 1). Rheumatoid arthritis was the most common, 59 cases in all were found in 1957–71. A combination of rheumatoid arthritis and LE cells was found in only 3 patients. LE cells were not investigated in all cases of rheumatoid arthritis.

Cases of scleroderma were distinguished by means of typical skin and muscle biopsy findings and clinical symptoms. One patient who had signs of scleroderma on skin and muscle biopsy but subsequently died of generalized lupus disease with typical lupus nephritis was autopsied at Malmö General Hospital. Professor J. Wahlen in Malmö provided diagnostic advice in several similar interesting cases.

Only 5 cases of dermatomyositis were found one with ovarian cancer as the underlying disease.

A total of 5 fully developed cases of hydralazine syndrome were found; the patients recovered on withdrawal of the drug.

A 60-year-old man illustrates typical case 1b. He had been treated with approximately 100 mg Apretin daily for 10 years. In Feb. 1971 he had developed arthralgia, fever, nose-bleed, microscopic hematuria, high ESR, polyclonal γ -increase, high ANF titre, positive RA test and pronounced immunoblast infiltration both in the spleen and liver and muscle biopsy findings compatible with SLE. A few months after withdrawal of Apretin the patient was clinically symptom-free and there was considerable normalization of both spleen and liver cytology as assessed by Professor N. Söderström. Corticosteroid treatment was not necessary.

Rheumat arthritis	257
Rheumatoid arthritis + LE-cell	3
Skin edema	11
Stomatitis	8
SLE	88
Hydralazine-induced SLE	5
Proliferative SLE	4
Thrombotic thrombocytopenic	1
Interstitial nephritis	2
Interstitial nephritis	3
Lupoid hepatitis	10
Polyarteritis nodosa	3
Polyarteritis nodosa	48
Goodpasture's syndrome	2

Fig. 1 Cases of SLE and related diseases hospitalized in the Departments of Medicine and Infectious Diseases, County Hospital, Halmstad in 1957-71

every Patients who may develop hydralazine lupus syndrome are slow acetylators with low *N*-acetyl transferase. This patient had long PTs for INH 5 h and 2 min and is thus a slow acetylator

Four patients with procainamide-induced lupus syndrome were found. All recovered upon discontinuation of the drug (in 1972-73 a further 4 cases were found)

Only occasional cases of autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura were diagnosed and no case of purpura by peribulbemia was seen

Chronic aggressive ('lupoid') hepatitis was distinguished by means of the clinical picture laboratory findings and cytology. All these patients except one were women with high ANF titres and positive LE cells. All had antibodies to glomeruli and smooth muscle suggestive of chronic aggressive hepatitis.

Cases of temporal arteritis and polymyalgia arterica were included in related diseases since these conditions may cause problems of differential diagnosis especially in the early stages of the disease

The incidence of definite SLE was found to be 17.6/year/1 mill from mid 1957 to mid 1964 and 16.2 from mid 1964 to mid 1971 in the Department of Medicine. The total SLE material selected according to the same principles from the Departments of Medicine and of Infectious Diseases in 1964-71 gave a higher incidence, however 29.7. The incidence of suspected SLE during the first 7 year period was 19/year/1 mill and during the following 7 year period 12.2

We found approximately the same age and sex distribution as in previous reports with a higher incidence in young and middle-aged women. In our material the mean age at onset of the first clinical symptoms was, however several years higher than in other reports. The mean age at onset in the Department of Medicine in group A was 46.5 and in group B 46.4 years. In the total material from both departments the mean age at onset in group A was 48 years. Fig. 2 illustrates age and sex distribution at onset of definite SLE.

Delay in diagnosis defined as the interval between the year of onset of symptoms and the year of diagnosis in group A was on an average 3.9 survival after onset 8.6 and survival after diagnosis 4.7 years. Fig. 3 illustrates the pronounced individual variations in the course of the disease

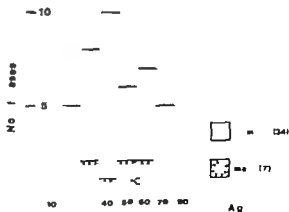


Fig. 2. Age and sex distribution at onset of definite SLE.

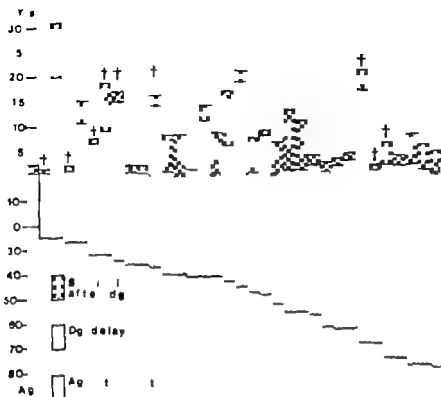


Fig 3 Course of 34 female patients in group A. Dg=diagnosis, †=death.

In the women of group A ($n=34$) The mean age at onset was 48 years the mean delay in diagnosis 4.9 years. The mean survival after diagnosis was 4.2 and after onset 9.1 years. Nine patients died, 4 of them from uremia. In group B ($n=25$) the mean delay in diagnosis was 10.6 years survival after onset 15.9 years and survival after diagnosis 9.3 years. Ten patients died, 2 of them from uremia.

Table I shows the percentage occurrence of various clinical manifestations in groups A and B. Figures show many similarities to Leon's larger material from Malmö (13) comprising 57 definite and 52 suspected SLE cases. Among the skin symptoms found in the present material was the chronic discoid type of LE exanthema, but acute or subacute skin conditions of morbilliform and scarlatiniform type and recurrent or tickle-like were more common. The typical butterfly exanthema was found in 39% of patients in group A. Purpura, which can be caused by thrombocytopenia or vasculitis, was diagnosed in only a few cases as also was loss of hair.

Joint symptoms in the form of arthralgia and often myalgia without definite objective findings

occurred and acute and subacute polyarthritis with localized swelling of the joints and restriction of movement, involving various small and large joints were also found. Chronic joint changes which could often not be distinguished from rheumatoid arthritis with typical radiographic arthritic and/or typical clinical symptoms, were often found. Lymphadenitis was diagnosed in only 24% of patients in group A.

Liver involvement was present in half of the cases i.e. either pronounced enlargement of the liver and/or pathological liver tests (positive thymol not included) and pathological liver cytology findings. Seventeen patients had pathological liver tests usually in the form of raised transaminases raised alkaline phosphatases sometimes raised bilirubin. Liver cytological investigations were performed in 11 patients in group A. One patient had slight steatosis during concomitant steroid therapy this being a non-specific finding. Two patients had very extensive steatosis, one of whom had pronounced enlargement of the spleen with pronounced blast cell infiltrations. Four patients had symptoms of active hepatitis with pronounced lymphoblast and immunoblast infiltration.

Table 1 Cumulative incidence (SE) of commonest clinical manifestations of SLE in the present study and in Leonhardt's material

	Present study		Leonhardt (13)	
	A (n=41)	B (n=25)	A (n=57)	B (n=57)
Cutaneous LE	78	28	60	35
Butterfly erythema	39	16	49	15
Purpura	5	0	19	17
Loss of hair	10	4	25	4
Arthropathy	90	80	93	92
Rheumatoid arthritis	29	16	18	35
Lymphadenopathy	24	0	46	28
Splenomegaly	20	12	23	25
Hepatomegaly	59	20	18	1
Hepatopathy			7	11
Primary lesions	46	20	60	44
Pleurisy	37	28	70	64
Myocarditis	32	28	28	15
Pericarditis			21	19
Raynaud's phenomenon	15	0	25	21
Leg ulcer + phlebitis	17	4	28	10
Sicca syndrome	12	8	11	31
Inter-iriditis	10	4	1	4
CNS lesions	7	8	11	8
Nephropathy	34	28	56	23
Anaemia	37	32	63	42
Leucopenia ($<3000/\text{mm}^3$)	32	4	77	56
Thrombocytopenia ($<100000/\text{mm}^3$)	17	4	32	17
Hyper- γ ($>1.45 \text{ g/100 ml}$)	81	72	90	90
($>2.8 \text{ g/100 ml}$)	63	24	68	69
LE cells	71	48	75	54
LE cells - RHB rosettes	85	84	-	-
ANF	96	55	-	-
Rheuma serology	44	39	41	66
Positive Coombs' test	12	4	18	2
False pos. syphilis serology	5	0	16	15
Fever	78	42	88	92
Drug reactions	34	0	53	31
Remitting course	63	48	81	85

One patient had pronounced bile duct proliferation. (One patient had extensive cirrhosis of the liver at autopsy.)

Chronic pneumonia not responding to antibiotics, pleurisy (often bilateral and recurrent) and various forms of cardiac involvement, above all perimyocarditis, occurred in 46-32% of the patients. In two cases classical endocarditis of Libman-Sacks type was found at autopsy. Raynaud symptoms occurred in 15% but no definitely positive cryoglobulin tests were found in this group. Among CNS symptoms found were cranial

nerve paralysis, convulsive seizures and mental confusion responding to treatment with cortisone.

Nephropathy was found in only one-third of the patients. In view of the frequent occurrence of concomitant infections and other kidney diseases we included only definite or strongly suspected cases comprising nephropathy with or without nephrosis and fatal glomerulonephritis with uremia. Serum complement investigations were performed in two patients with active glomerulonephritis and uremia and the titres were found to be low. Two patients with lupus nephritis and pre-uremia or uremia were subsequently admitted to the University Clinics in Lund and Gothenburg respectively.

The occurrence of anaemia, leucopenia and thrombocytopenia was low compared to other reports. The criteria for anaemia in women was $\text{Hb} < 10 \text{ g/100 ml}$. A large number of women had Hb values between 10 and 11 g/100 ml.

Hypergammaglobulinemia $> 1.45 \text{ g/100 ml}$ occurred in 83 and 77% and $> 2.8 \text{ g/100 ml}$ respectively. γ -globulin was $> 2 \text{ g/100 ml}$. All of these patients had increased polyclonal γ -globulin with the exception of two who had discrete M components of benign type.

One elderly man with familial disposition for joint pains had on admission symptoms suggestive of SLE, bilateral arthropathy, tiredness, loss of weight, moderate enlargement of the liver, anaemia, high ESR, typical LE cells in one test, and highly positive ANF titres in three samples. Immunoelectrophoresis showed, however, monoclonal γ -band and IgG L myeloma. Radiographic skeletal changes typical of myeloma developed subsequently. The patient did not fulfil the criteria for suspected SLE and was excluded from the group.

Of the cases in group A 71% and in group B 48% were found to be LE cell positive, most of them investigated according to the Zinkham-Conley method. The figures rose to 85 and 84% when round hematoxylin bodies of homogeneous type and rosettes were included. All patients from the Department of Medicine in group A diagnosed after 1964 had positive ANF titres. ANF titres were investigated in 11 of 13 patients from the Department of Infectious Diseases, one of them having a negative titre. Investigated once and the other ten positive. The incidence of positive ANF titres in the total material from both

putments was 96%. In most cases the laboratory concerned considered the titre highly positive and suggestive of SLE. Only 60% of the patients in group B had positive ANF titres. Most of the patients with negative titres received concomitant corticosteroid therapy. The ANF titre was found to fluctuate considerably during the course of the disease. One patient with lupus nephritis became ANF-negative during the fulminant phase of the disease during which treatment with azathioprine and cortisone was given. Some of the other patients had high titres without signs of active disease.

Different kinds of tests for the presence of rheumatoid factors were used. They generally yielded weakly to moderately positive results, thus a non-homogenous group showing positive figures in 46 and 39% respectively. Only a few cases with positive Coombs' test were found in group A and these had definite hemolysis. The figures for other non-specific antibodies and false positive results of serological tests for syphilis performed in only a few cases were low compared to other series. Fever and recurrent infections occurred frequently.

Many interesting constellations of symptoms were observed. Some patients in group B had had symptoms of rheumatoid arthritis for many years and had subsequently been admitted to hospital with refractory pleurisy, symptoms of nephritis or hemolytic anemia suggestive of progression to SLE. This group included several other patients with interesting combinations of diseases such as the occurrence of rheumatoid arthritis, hyperthyroidism, myasthenia gravis and histamine-refractory achlorhydria as well as LE cells in one and the same patient. Another patient had the

same clinical combination and in addition pronounced vasculitis with formation of skin ulcers which healed after treatment with cortisone. Group B also included two patients with chronic discoid lupus erythematosus. One of them also had certain manifestations suggestive of SLE, while the other had only this cutaneous manifestation, LE cells and persistent increase in polyclonal γ -globulin. One patient in group A had in addition to rheumatoid symptoms vasculitis, butterfly exanthema, leucopenia and LE cells highly suspect chronic lymphatic leukemia. Most of the patients in group B had a more benign and chronic course and the delay in diagnosis was several years longer.

Polypharmacy has been widespread. Drug reactions occurred in 39% of the patients in group A. Four had penicillin reactions, one anaphylactic shock after injection of penicillin, three skin reactions after treatment with sulphonamides, one developed a skin reaction after treatment with iodine and one after treatment with acetyl salicylic acid. Another patient developed a skin rash, possibly due to the colouring matter in Deltacortril® tablets and one had suspected erythromycin erythema. In one case SLE developed shortly after treatment with gold. We assess remission of the disease to have occurred in slightly more than half the cases. These figures are difficult to judge however.

As regards therapy salicylates were given to practically all patients. Antimalarials were given to several patients with joint symptoms and to patients with chronic discoid lupus erythematosus. Cortisone was given to 36 of the 41 patients in group A, 18 required continuous treatment, usually with low doses, 5-7.5 mg Prednisolon® daily. A dramatic improvement was noted in connection with large doses of cortisone in patients with severe cardiopulmonary manifestations, CNS syndrome, hemolytic attacks and thrombocytopenia. One woman in group B with pleuropneumonia and pericarditis of SLE type with much impaired general condition and high fever recovered spontaneously and was symptom-free for several years except for raised ESR. Azathioprine was given together with cortisone to only four patients, three of whom had lupus nephritis. One patient improved with reduced proteinuria and normalization of serum complement. In another patient with nephritis and uremia, treatment with azathioprine and cortisone brought only a transient improvement and in a third patient no improvement was observed.

Nine patients in group A died, five from uremia, one from leucopenia and fever, one from furunculosis in connection with fracture of the femur, one from gastrointestinal bleeding and one from myocardial infarction. Ten patients in group B died, two from uremia, two from heart failure due to perimyocarditis, one from myocardial infarction, one from complete AV block and septic arthritis, one from gastrointestinal bleeding, one from breast cancer with metastasis and one from generalized fungal infection. One patient died at home, possibly from bleeding gastric ulcer.

DISCUSSION

The present study shows considerable similarities regarding clinical and laboratory findings and course to previous SLE series, especially the investigations published by Leonhardt (13). In the present study the definite cases of SLE were defined according to principles generally accepted by many authors and confirm the clinical entity of definite SLE. Many borderline cases were found, however between suspected SLE and related diseases, some of these later showing typical signs of definite SLE. Leonhardt (14) demonstrated the most important manifestations of SLE with regard to incidence in his own and five other series. He found considerable variations in incidence due to differences in the primary patient series, in criteria for selection and in definitions of symptoms such as leucopenia, etc. The frequency of SLE compared to that of related diseases in this study shows many similarities to the investigations of Dubois (4) and Kurland *et al.* (10).

In the present study three patients were found with rheumatoid arthritis and LE cells. This combination is considered to be a fairly benign condition, very rarely progressing to active lupus disease. Lupus nephritis according to Dubois (4) has however been found in renal biopsies among patients in this group.

Patients with hydralazine syndrome could be distinguished from SLE patients by recovering on withdrawal of the drug; besides these patients belong to the slow acetylator group.

Four patients had procainamide-induced lupus syndrome all of whom recovered upon discontinuation of the drug. Henningsen (8) found in a prospective study that more than half of the patients given procainamide developed positive ANF often with increasing titres. Some of these may progress to lupus syndrome. Henningsen therefore recommends ANF control at intervals of 2-3 months. Serious kidney injuries have not been reported either in procainamide or hydralazine-induced lupus syndrome.

Among the SLE manifestations nephropathy was found in only one-third of the patients. The incidence of lupus nephritis was probably considerably higher but in only a few cases were biopsies and thorough autopsies performed. The incidence of nephropathy is higher in most other SLE series published. Thus according to Pollak *et al.*

(19) renal involvement is found at autopsy in at least 75% of patients with SLE. Lymphadenopathy was diagnosed less frequently in this material than in many others probably due partly to inadequate clinical diagnosis. Hepatopathy was commonly found in this study occurring in 49% in group A probably due to cytological and repeated laboratory investigations. Anemia, leucopenia and thrombocytopenia were not so common in this as in Leonhardt's study. Hb concentrations below 10 g/100 ml in females were considered anemia. Hb values between 10 and 11 g/100 ml were very often found however. Many patients had leucocyte counts just above 3000/mm³ and a platelet count just above 100 000/mm³.

LE cell positive cases increased from 71 to 84% in group A and from 48 to 84% in group B when round hematoxylin bodies of homogenous type and rosettes were included. According to Dubois' extensive investigations (4) the presence of round hematoxylin bodies and rosettes in several samples is highly suggestive of SLE. False positive syphilis serology was found only in two cases. Unfortunately this test was performed in only a few cases.

Polypharmacy has been widespread and resulted in many side-effects, one patient developed lupus shortly after treatment with gold. Exacerbation of lupus disease after gold therapy has been reported in other series. Seven patients had side-effects to antibiotics. Presumably one ought to be more restrictive in treatment of these cases with sulphonamides and penicillin and indeed drugs in general.

As regards therapy salicylates were given to practically all patients, one of them showing skin reaction in connection with acetyl salicylic acid treatment. Antimalarials were given without any side-effects and most of the patients were given cortisone with often dramatic improvement, only four patients received azathioprine.

Approximately the same age and sex distribution was found in this study as in previous reports with a higher incidence in young and middle aged women. In this study the mean age at onset was however several years higher than reported by others. In Leonhardt's material for example, the mean age at onset in group A was 36.6 years and in group B 39 years as compared to 43 and 46.4 years in the present study. Larsen (11) found that 70.8% of the women first fell ill between 20

and 50 years of age and the disease was diagnosed between 40 and 60 years in 70.5% of cases.

The delay in diagnosis in group A in this study was 4.9 years about .6 years shorter than that reported by Leonhardt. In group B the delay in this study was 10.6 years about 3.1 years longer than in Leonhardt's material.

The mean survival time after diagnosis was 4.7 years in group A and 5.3 years in group B. However the follow-up time is short in the present study in several cases only a few years. As expected there was a high mortality due to lupus nephritis of 66 SLE patients 17 died six of them from uremia. Leonhardt (16) estimated the long-term prognosis for SLE patients and reported a 5-year survival after diagnosis in 70.3% and a 10-year survival in 51.3%.

It is noteworthy that the incidence tends to increase when the cases from the Departments of Infectious Diseases and Medicine are added together it reaches 29.7/year/1 mill inhabitants (mean incidence during the years 1964-71). The population increased during the same period from 99 000 to 111 700. Leonhardt found the incidence between 1955 and 1961 to be 10.3 in Malmö with 90 000 inhabitants. The incidence from Scania except Malmö was 5.2. Larsen (11) found the incidence in central and western Norway to be similar to that in Leonhardt's study varying between 4.1 in 1961 and 10.3 in 1962. His material was collected during the years 1959-63. The increasing incidence was considered to be mainly related to the introduction of the LE cell test. Siegel et al. (22) estimated the approximate incidence in New York City since 1955 to be 10/year/10 inhabitants. In a later investigation Siegel

(21) reported higher incidence figures. In cases from New York City and Jefferson County, Alabama in 1956-65 these authors found a total incidence in New York of 18.5 and in Alabama of 9.9/1 mill. A considerably higher incidence was found in coloured women 75.4 in New York and 26.5 in Alabama, probably due to ethnic factors. Among white women in New York City the incidence was 25.7 and in Alabama 10.6. Siegel considers the increasing incidence of SLE to be due mainly to improved diagnostic resources. He also found increasing prevalence figures due to earlier diagnosis and improved therapeutic resources. Conclusions made from the present study

the limitations of which are recognized support these opinions.

The increasing incidence of SLE is probably due to improvements in diagnosis but the possibility of SLE becoming more frequent, possibly as a result of various environmental factors bacterial and viral infections together with polypharmacy and other chemical and physical triggering factors cannot be excluded.

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Table I *Laboratory data before and during treatment with prednisolone and cyclofenil*

After 1 1/2 months the prednisolone dose was reduced to 5 mg/day and the cyclofenil treatment started. After a further 11 months prednisolone treatment was stopped and the cyclofenil dose doubled

	Before treatment	After 2 1/2 mo prednisolone (20-30 mg/d)	After 6 mo cyclofenil (300 mg/d) + prednisolone (5 mg/d)	After 4 mo cyclofenil (600 mg/d)
Hb (g/100 ml)	13.1	13.2	13.7	14.6
RBC/mm ³ 10 ⁶	4.8	4.3	4.4	5.0
WBC/mm ³	7 000	6 000	7 400	6 000
Platelet 1 000/mm ³	247	251	250	327
ESR (mm/h)	57	31	29	20
Serum creatinine (mg/100 ml)	0.9	0.8	0.9	0.9
Serum calcium (mEq/l)	4.9		4.8	4.6
Serum albumin (g/100 ml)	3.9	4.2	3.9	4.2
Serum haptoglobin (mg/100 ml)	40	170	155	1.5
Serum bilirubin (mg/100 ml)	0.6	0.4	0.6	0.4
Thymol (U)	1.0	0.9	1.5	1.4
Alkaline phosph (U)	1.0	1.2	1.6	1.6
GOT (U)	20	12	56	33
GPT (U)	24	11	72	46
LDH (U/ml)	283	201	290	281
Rheumatoid factor (latex fixation test)	Pos. 1/640		1/320	
Serum γ -globulin	Elevated		Elevated	Normal
ANF	Pos. 1/400	Pos. 1/400	Pos. 1/100	Neg.

thritic changes together with the stiffness of the skin resulted in restrictions of head movements and inability to elevate the arms above the horizontal plane.

In Feb. 1973 the patient was admitted to the Department of Medicine. In addition to the physical changes X-ray examination revealed reduced esophageal mobility. Laboratory investigations showed high ESR, moderate decrease of serum albumin, increased serum haptoglobin level, normal liver and kidney functions. There were positive titres of the latex fixation test and of antinuclear factor (ANF) (Table I).

Conventional prednisolone therapy was started with one of 20-30 mg/day. Simultaneously intense physiotherapy by daily exercises and hydro-baths were against the arthritic changes. After 2 1/2 months edematous therapy no subjective or objective improvement could be observed and the clinical condition continued to progress. At this time the prednisolone dose was reduced and Sexovid (cyclofenil) treatment was started with daily dose of 100 mg three times daily.

After some weeks the patient noticed considerable improvement in several respects. The affected skin areas became gradually softer, less stiff and more elastic, especially in the face, neck and chest. The joints were swollen and painful and the mobility increased. After 6 months treatment the beneficial action of the drug and its lack of side-effects led to an increase of the dose to 200 mg three times a day. The patient was observed further on the higher cyclofenil dose over 5 months. From May to Nov. 1973 the pred-

nisolone dose was kept at a very low level (5 mg/day) and since Nov. the patient has received no prednisolone at all.

The results of some laboratory investigations are shown in Table I. It can be seen that during the 1 year observation period there were no changes in the standard haematological values. ESR and the serum haptoglobin levels show a gradual decrease. In contrast, the cyclofenil treatment caused a moderate increase in the serum GOT and GPT levels indicating

possible effect of the drug on liver cells. However, despite further uninterrupted cyclofenil administration the transaminase levels decreased again. Recently the liver cells appeared microscopically normal in a biopsy material and the galactose loading test was found to be normal. The titre of rheumatoid factor decreased and the ANF disappeared, possibly due to the corticosteroid treatment.

The improvement in the patient's respiratory function over the last treatment period is shown in Table II. It is rather difficult to record small changes in joint function objectively but an attempt was still made to study mobility in some joints especially involved. Table III demonstrates that a certain increase in the mobility of the shoulders, wrists and the small interphalangeal joints had been achieved. The stiffness of the neck diminished and the gaps between the teeth had increased. The drug was apparently well tolerated during the whole observation period and apart from the moderate increase in transaminase levels no side-effects were noticed.

Table II. Effect of treatment with 600 mg cyclofenil daily on the respiratory function measured by dynamic spirometry

MVV = maximum voluntary ventilation, PEF = peak flow FEV = forced expiratory volume

	Normal value	Before dose increase to 600 mg		After 4 mo on 600 mg	
		Patient value	% of normal	Patient value	% of normal
MVV (l/min)	149	90	60	118	79
MVV ₂₅ (l/min)	122	90	74	109	89
PEF (l/min)	580/425	475		560	
Vital capacity (l)	4.8	3.7	77	3.9	81
FEV (l)	3.5	2.8		2.9	

DISCUSSION

There are several factors which make it difficult to evaluate the possible therapeutic effect of a new drug in systemic sclerosis. Firstly the generalized forms of the disease are rather rare. Secondly the clinical course and symptomatology as well as the character of the pathological changes differ from case to case. Thirdly spontaneous periods of standstill and remissions are not uncommon. Moreover specific laboratory parameters

that might indicate the progress or regress of the disease are lacking.

In the present case the observation time was made rather long in order to exclude the probability of spontaneous remission. An attempt was also made to objectively quantitate respiratory function and joint mobility in the affected joints but it is obvious that the pathological change before treatment must be rather advanced to permit the recording of significant improvements. There fore in mild cases and in the early phase a suitable biochemical parameter would be needed for evaluation of small therapeutic effects. The present case was advanced before treatment and cyclofenil caused measurable improvement of the kin and joint changes as well as of the lung function. However it is also of interest that the laboratory profile became somewhat altered during the treatment. The decrease in some immunological titres and the fall of the serum haptoglobin level may be due to prednisolone administration in the initial stage but the changes appeared in connection with the cyclofenil treatment and seem to be permanent despite a long prednisolone-free period. During the experimental evaluation of cyclofenil however no anti-inflammatory effect was detected (2).

Nothing is known about the mechanism by which cyclofenil acts upon the sclerodermic tissue. It is unlikely that the pituitary-inhibiting properties would play a role in producing the beneficial effects (1, P55). It is rather possible that this drug, in analogy with conventional estrogens, in some way alters the metabolism of collagen and proteoglycans. A variety of such estrogenic action has previously been demonstrated

Table III. Function of some joints measured before and after cyclofenil treatment

Until 8 months 300 mg, after 8 months 600 mg daily

	Before treatment	After 8 mo treatment	After 1 y treatment
Maximal elevation of the arms in the sagittal plane	120°	135°	145°
Fast closure: long-finger distals (cm)			
Right	3.0	1.5	1.0
Left	2.0	1.0	0.5
Dorsal flexion of the wrist joint			
Right	5°	7°	10°
Left	5°	10°	15°
Palmar flexion of the wrist joint			
Right	5°	10°	15°
Left	10°	10°	15°
Maximal antero flexion of the trunk, distance between fingertips and floor (cm)	30	14	15
Maximal dorsal flexion of the head, distance between hyochra jugularis and lower tooth row (cm)	17	17.5	20

in animal experiments by many investigators (4, 7, 9, 10, 13). Recent studies indicate that the rate of collagen and proteoglycan synthesis in sclerodermic skin is rather well correlated with the severity of the disease (14). Thus incubation experiments using skin biopsy specimens and radioactive precursors at different intervals during cyclofenil treatment seem to be a possible way to evaluate therapeutic effect. Such objectively controlled studies have now been started in our department on a larger group of PSS patients. Using doses similar to those in the present case cyclofenil might be somewhat estrogenic and inhibit gonadotrophin secretion (2, 11, 12). Therefore it seems convenient to test further the effects of the drug in a female material where estrogenic side-effects would be of no significance.

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RECURRENT ATTACKS OF HEMOLYTIC ANEMIA THROMBOCYTOPENIA AND UREMIA—HEMOLYTIC UREMIC SYNDROME?

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Abstract. The case history of a 3-year-old boy with 6 relapses of hemolytic anemia, thrombocytopenia and uremia is reported. Between the attacks the glomerular filtration rate was normal and there was no proteinuria. The possible relationship to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura is discussed.

In 1925 Moschowitz (10) described thrombotic thrombocytopenic purpura (TTP). The disease was characterized by fever, purpura, hemolytic anemia, neurological manifestations and sometimes uremia. The typical histological lesion was occlusion of the small arteries in multiple organs caused by disseminated intravascular coagulation. Thirty years later Gasser et al (7) reported the hemolytic uremic syndrome (HUS) as a new symptom complex including hemolytic anemia, thrombocytopenia, neurological manifestations and severe uremia. The disease was always lethal and autopsy showed bilateral cortical necrosis in the kidneys and dispersed thrombotic microangiopathy. Gasser et al emphasized that this new syndrome differed from the TTP mainly in the severe kidney damage.

The etiology of these syndromes has been widely discussed but remains unknown. Before treatment with dialysis, heparin and corticosteroids the course was nearly always fatal. The prognosis at present may have changed and total remission has been reported (9).

As regards the prognosis we therefore find it important to report on a case of a 3-year-old boy with total remission in kidney function after six relapses of hemolytic anemia, thrombocytopenia and uremia.

CASE REPORT

A 3-year-old boy was admitted to the hospital in Nov. 1971 (Fig. 1) with 2 days' history of fatigue, nausea, vomiting,

jaundice of the sclerae, dark urine, petechiae and ecchymoses on the legs ill accompanied by common cold. He had a moderate enlargement of the liver and small elevations of the transaminases, which both vanished during the hospitalization.

Analytes showed Hb 6 g/100 ml, platelets count 5000/mm³, reticulocytes 1%, osmotic resistance normal, haaptoglobin slightly decreased, leucocytes 100/mm³. The peripheral blood smears showed no siderotic areas of macrocytosis and poikilocytosis with numerous Burr cells. Bone marrow aspirate revealed no signs of leucemia. Clotting time 3 h, menorrhagia time more than 10 min, prothrombin 64%. All other coagulation factors normal. Serum creatinine 2.6 mg/100 ml, BP 115/65, protein content in urine 5 g/24 h. Urine sediment 40-50 erythrocytes, 6-8 leucocytes, urine protein positive. Total serum bilirubin 3.6 mg/100 ml, SGOT and SLDH grossly increased, alkaline phosphatases normal. Liver biopsy normal, kidney biopsy unsuccessful. The following tests were all negative: Coombs test, antiglobulin consumption test, antinuclear factor, LE cells, WR, Paet-Banquet isoxanthinase, herpes virus, leptospira, influenza complement, adenovirus, streptococcal MG, cytomegalovirus, Wad, polio, echo virus in feces. Urine mercury, arsenic, lead, copper, zinc and iron all normal.

The patient was treated with two blood transfusions and recovered completely within 7 days. Kidney function returned to normal and proteinuria vanished.

The patient remained healthy until 10 months later when he was readmitted to the hospital with the same symptoms of dark urine, jaundice, fever, petechiae and ecchymoses. Laboratory investigations as above. He was treated with blood transfusions, platelet transfusions and furosemide. He recovered slowly to normal kidney function and proteinuria disappeared.

Six months later he was readmitted again with symptoms as earlier. On this occasion the kidney function was severely decreased and the patient had to be transferred to the Nephrological Department for peritoneal dialysis. Due to the dialysis, short treatment with heparin was interrupted. The patient was given prednisone 40 mg/day. After two peritoneal dialyses he recovered slowly and the prednisone therapy was withdrawn.

Kidney biopsy examined by Dr C. Bruus, showed glomerulonephritis proliferativa, TTP (Mos-

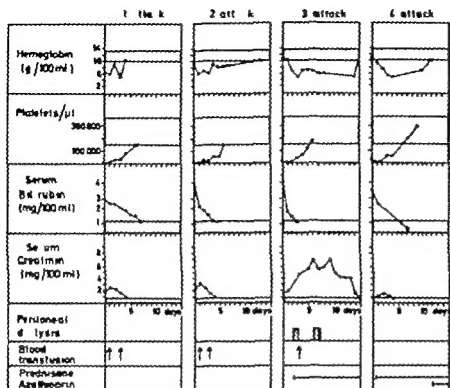


Fig. 1 Laboratory data from the first four hospitalizations.

and is illustrated in Fig. 1. In addition to the former laboratory investigation on this occasion a coagulopathy was found. Free Hb in serum 161 mg/100 ml in urine 404 mg/100 ml. The antiglobulin consumption test was now positive. Erythrocyte glucose-6-phosphat dehydrogenase activity normal. I urography normal.

Again he remained healthy with normal kidney function and no proteinuria until 9 months later when a new relapse took place with symptoms as earlier. The patient was immediately treated with prednisone 40 mg/day and recovered quickly without need of dialysis. Treatment with azathioprine was started as long-term therapy and ultimately the prednisone dosage was reduced.

During further follow-up (not indicated in Fig. 1) the patient had 1 more hemolytic episode with temporary affection of the kidney function and the azathioprine treatment has now been withdrawn. The kidney function is again normal and no proteinuria remains.

DISCUSSION

The case history of a 3-year-old boy with six relapses of hemolytic anemia, thrombocytopenia and uremia and normal kidney function and no proteinuria between the attacks is presented. Each attack fulfills the typical clinical and pathological features of the hemolytic uremic syndrome but to the author's best knowledge recurrent relapses after

total remission of the HUS have not previously been reported.

The etiology of the HUS is still uncertain. Many authors have discussed the possible relationship to a preceding virus infection. Gianantonio et al. (8) suggest an epidemic appearance and have isolated a conceivable viral agent in 8 patients during the acute stage of HUS. In 36 of 55 patients they found complement fixing antibodies to this virus. Ray et al. (11) demonstrated in 2 patients a possible connection between infection with coxsackie virus B positive serology and the acute stage of HUS. Furthermore in 1970 Ray et al. (12) found serological evidence of infection with enterovirus (coxsackie virus) in 7 of 9 patients with HUS. We have not been able to demonstrate any agent responsible for the outbreak of HUS in our patient but every attack was preceded for a few days by signs of a common cold.

Other possible etiological mechanisms have been reported e.g. estrogens (1), drugs (15), endotoxin reactions (Schwartzman's phenomenon) (14), decreased glucose-6-phosphate dehydrogenase activity (favism) (4), sickle cell (3) but the problem is still open for further investigation.

HUS and TTP are commonly considered as two distinctly separate diseases although the clinical and pathological findings often overlap and one may

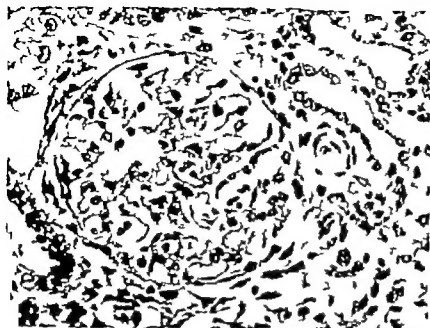


Fig 2 Glomerular corpuscle showing open tufting capillaries, hypercellularity and increased vascularity. The arterial (arrow) greatly damaged to character the thickening of the wall and obliteration of the lumen.

find the typical TTP microthrombi in patients suffering from both conditions. The clinical picture of each attack in our patient shows the typical pattern of HUS while the pathologist describes the findings in the kidney biopsy as TTP.

From a review of the literature one may consider the two syndromes as parts of one greater entity (5) the HUS being most often found in children while the TTP is a disease of adult patients still with many exceptions. This view is not yet commonly accepted.

The prognosis of both syndromes is poor (5) but in a few materials a low mortality has been reported (6). The treatment is still symptomatic with blood transfusions and dialysis in the uremic stage while no authors have been able to show a striking effect on survival after treatment with heparin, corticosteroids or immunosuppressive agents (azathioprine). Full recovery has been reported (6, 13) as well as a long chronic course with death in terminal uremia (2, 8) but recurrent relapses with normal kidney function and no proteinuria between the attacks have not been reported earlier.

In our patient the immunosuppressive therapy had no effect since new attacks broke out during this treatment.

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